VOLUME 32 FEBRUARY 1954 NUMBER 1

Canadian Journal of Zoology

Editor: E. HORNE CRAIGIE

Published by THE NATIONAL RESEARCH COUNCIL
OTTAWA CANADA

CANADIAN JOURNAL OF ZOOLOGY

(*Formerly Section D, Canadian Journal of Research*)

The CANADIAN JOURNAL OF ZOOLOGY is published bimonthly by the National Research Council of Canada under the authority of the Chairman of the Committee of the Privy Council on Scientific and Industrial Research. The National Research Council publishes also: *Canadian Journal of Biochemistry and Physiology, Canadian Journal of Botany, Canadian Journal of Chemistry, Canadian Journal of Physics, Canadian Journal of Technology.*

Matters of general policy concerning the Council's journals are the responsibility of a joint Editorial Board consisting of: the Editors of the journals; members representing the National Research Council of Canada and the Royal Society of Canada and two other scientific societies; and two ex-officio members.

EDITORIAL BOARD

Editors of the Journals

D. L. BAILEY, *University of Toronto*
J. B. COLLIP, *University of Western Ontario*
E. H. CRAIGIE, *University of Toronto*
G. A. LEDINGHAM, *National Research Council*
LÉO MARION, *National Research Council*
G. M. VOLKOFF, *University of British Columbia*

Representatives of the National Research Council

C. W. ARGUE (Chairman) *University of New Brunswick*
G. E. HALL, *University of Western Ontario*
T. THORVALDSON, *University of Saskatchewan*
W. H. WATSON, *University of Toronto*

Representatives of Societies

D. L. BAILEY, *University of Toronto*
Royal Society of Canada
E. H. CRAIGIE, *University of Toronto*
Royal Society of Canada
H. G. THODE, *McMaster University*
Chemical Institute of Canada
T. THORVALDSON, *University of Saskatchewan*
Royal Society of Canada
G. M. VOLKOFF, *University of British Columbia*
Royal Society of Canada
Canadian Association of Physicists

Ex officio

LÉO MARION (Editor-in-Chief)
National Research Council
H. H. SAUNDERSON,
National Research Council

Manuscripts should be addressed to:

Dr. Léo Marion,
Editor-in-Chief,
Canadian Journal of Zoology,
National Research Council,
Ottawa 2, Canada.

For instructions on the preparation of manuscripts and illustrations, see **Notes to Contributors** (inside of back cover).

Subscriptions, renewals, and orders for back numbers should be addressed to:

Division of Administration,
National Research Council,
Ottawa 2, Canada.

Subscription rate: $3.00 a year; single numbers: 50 cents. Remittances should be made payable to the Receiver General of Canada, credit National Research Council.

VOLUME 32 1954

Canadian Journal of Zoology

Editor: E. HORNE CRAIGIE

Published by THE NATIONAL RESEARCH COUNCIL
OTTAWA CANADA

Canadian Journal of Zoology

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOLUME 32 FEBRUARY, 1954 NUMBER 1

OXYGEN UPTAKE OF MUSCLE HOMOGENATES FROM THREE SPECIES OF INSECTS, IN THE PRESENCE OF ADDED SUCCINATE, CYTOCHROME *c*, AND PHOSPHATE[1]

BY WILLARD R. ALLEN[2] AND A. GLENN RICHARDS[3]

Abstract

The oxidation of sodium succinate by muscle homogenates prepared from the leg muscles of adult cockroaches [*Periplaneta americana* (L.)] and thoracic muscles of adult beetles (*Tenebrio molitor* L.) and adult flies (*Sarcophaga bullata* Parker) was studied. Oxygen consumption at 30° C. was measured in standard Warburg flasks. The standard conditions required for optimum activity were first established for each species studied. Activity was highest in the muscle of *T. molitor*, intermediate in *S. bullata* and males of *P. americana*, and lowest in females of *P. americana*. The difference between the sexes of *P. americana* was highly significant. A difference associated with sex was not observed in the other species examined. Significant differences attributable to age were not observed for *P. americana* or *T. molitor* adults, but a systematic increase in activity was observed during the early adult life of *S. bullata*; this was associated with the development of the thoracic muscle.

Introduction

Studies on the modes of action of insecticides have shown that it is desirable to study the deviations in physiological and metabolic processes that result in the symptoms of toxic action, and death. Several authors (3, 5, 9, 11, 12, 19) have obtained evidence that some of the enzyme systems of insects are significantly inhibited by insecticides. As a prelude to any study of inhibition by insecticides it is desirable to ascertain the conditions for enzyme determination in the species to be used. The present study supplies data for the American cockroach, *Periplaneta americana* (L.), the yellow mealworm, *Tenebrio molitor* L., and a species of fly, *Sarcophaga bullata* Parker, and reports differences among them.

[1] *Manuscript received February 18, 1953.*

Contribution No. 3066, Division of Entomology, Science Service, Department of Agriculture, Ottawa, Canada, and Paper No. 2914, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul 1, Minnesota. Based on part of a thesis submitted by W. R. Allen to the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Details on insect rearing, making activity measurements, as well as a review of the literature, may be found there.

[2] *Associate Entomologist, Field Crop Insect Section, Laboratory of Entomology, Brandon, Man.*

[3] *Professor, Division of Entomology and Economic Zoology, University of Minnesota.*

[The December, 1953, number of this Journal (Can. J. Zoology, 30 : 547-589. 1953.) was issued December 11, 1953.]

Methods

Muscle was dissected in cold saline solution (18) (sodium chloride, 10.93; potassium chloride, 1.57; calcium chloride, 0.85; magnesium chloride, 0.17; sodium bicarbonate, 0.17 gm. per liter of glass-redistilled water). The gut was carefully removed to avoid contamination by the contents. The cockroach was dissected by the method of Barron and Tahmisian (1). For the flies the large, indirect flight muscle and other thoracic muscles were removed. The beetles were similarly dissected, but for these both flight and leg muscle was used. Special care was exercised to free the muscle from other tissues. The wet muscle, ranging from 75.0 to 150.0 mgm., was weighed to ± 1.0 mgm. on a Roller–Smith torsion balance.

A 5% muscle homogenate in saline solution was prepared by a three-minute homogenization in a Potter–Elvehjem glass homogenizer at 1500 r.p.m. (13). The homogenizer was chilled before use and kept partially immersed in ice water during homogenization and until the preparation was used. Muscle to be frozen and stored before homogenization was placed in a vial containing 1.0 ml. of saline and immersed in a mixture of dry ice and acetone for about two minutes before placing in a deep freezer.

The standard Warburg technique was used to measure oxygen consumption at 30° C. following a slight modification of the method of Schneider and Potter (16). The gas phase was air, carbon dioxide was absorbed by potassium hydroxide–papers in the center well, and the flasks were shaken at a rate of 84 oscillations per minute. Flasks of approximately 15.0 ml. capacity were charged with 3.0 ml. of fluid contents. The quantity of tissue added, 2.5 to 5.0 mgm. (wet weight) per ml., was within the range in which oxygen uptake was proportional to tissue concentration. A sodium dihydrogen phosphate – sodium hydroxide buffer was used, cytochrome *c* was obtained from General Biochemical Inc., and sodium succinate was purified by a method previously described (14). Other chemicals were reagent grade.

After temperature equilibration, the contents of the reaction chamber were allowed to mix and re-equilibrate, then five-minute readings were taken for 35 minutes. For calculating data, the first 20-minute period was used. The pH was checked at the end of each run; change was negligible.

Total nitrogen was determined by the method of Ma and Zuazaga (10); duplicate 0.2 ml. samples of 5% muscle homogenate were used. The maximum difference between duplicate samples was ± 0.6 and $\pm 1.7\%$ for male and female cockroaches, respectively. However, the coefficient of variation between individual samples from the species examined ranged from 7 to 17%. Nitrogen values were determined for all tissue samples used except for some early runs, where average values were calculated as stated in the relevant tables and figure legends.

Results

Standard Conditions for Maximum Activity

Starting with the assay method found satisfactory for vertebrate tissues (16) concentrations of tissue, substrate, cytochrome *c*, phosphate buffer, and hydrogen ions were varied systematically. Data obtained for *P. americana*, in the age group of 10 to 20 days, are shown in Figs. 1–5. These data indicate that concentrations desirable for muscle homogenate of the male of *P. americana* are: 7.5 mgm. of fresh muscle, 0.025 to 0.05 *M* sodium succinate, 1.0×10^{-5} *M* cytochrome *c*, 0.025 to 0.05 *M* phosphate at a pH of 7.1 to 7.6. Conditions for maximum activity were similar for female muscle except that a higher tissue concentration (15.0 mgm.) was needed.

Less extensive tests showed that optimum conditions for activity are: for *T. molitor* muscle (15.0 mgm. of fresh tissue) 0.025 to 0.075 *M* sodium succinate, 0.025 *M* phosphate at pH 7.4, and 1.5 to 3.0×10^{-5} *M* cytochrome *c*; for *S. bullata* muscle (7.5 mgm. fresh tissue) 0.05 *M* sodium succinate, 0.05 *M* phosphate at pH 7.4, and at least 1.5×10^{-5} *M* cytochrome *c*.

In contrast to vertebrate tissue preparations (16, 8), calcium and aluminum ions, each added at 4.0×10^{-4} *M*, did not activate the systems present in

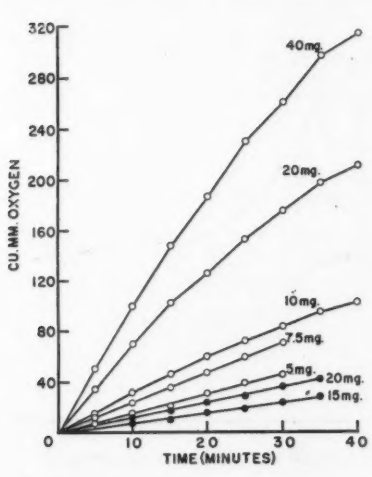


FIG. 1

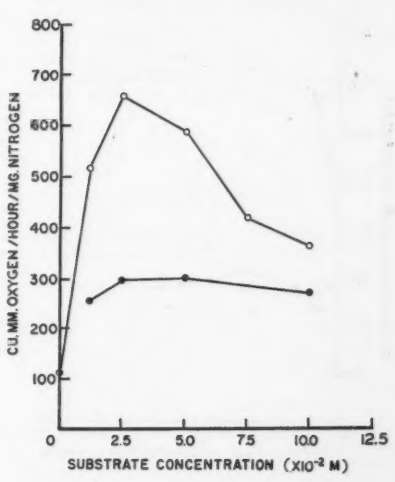


FIG. 2

FIG. 1. The effect of tissue concentration (fresh weight) on the oxidation of succinate at 30° C. by cockroach muscle, as a function of time. 0.05 *M* phosphate buffer at pH 7.4–7.5: 0.05 *M* sodium succinate. Open circles, male, 1.0×10^{-5} *M* cytochrome *c* added. Numbers of determinations in parentheses: 40 mgm. (1), 20 mgm. (4), 10 mgm. (2), 7.5 mgm. (7), and 5 mgm. (1). Solid circles, female, 7.5×10^{-6} *M* cytochrome *c*. Duplicate determinations. FIG. 2. The effect of substrate concentration on the oxidation of succinate at 30° C. by cockroach muscle. 0.05 *M* phosphate buffer at pH 7.4–7.6. Open circles, male, 1.0×10^{-5} *M* cytochrome *c*. Numbers of determinations, points left to right: 2, 6, 6, 6, 6, 4. Average nitrogen value used. Solid circles, female, 7.5×10^{-6} *M* cytochrome *c*. Numbers of determinations, points left to right: 2, 5, 5, 4. Average nitrogen value used first series, determined second series.

the muscle of *P. americana* or *T. molitor* when phosphate concentration was optimal. However, nicotinamide (0.01 *M*) and coenzyme I (5.0×10^{-4} *M*), both in excess, inhibited the system of the male and the female of *P. americana* by 45% and 30%, respectively, and calcium (4.0×10^{-4} *M*) seemed to antagonize this inhibition slightly (5%) in the male (7, 17).

The activity in homogenates prepared from frozen muscle, stored in a frozen state for 28 to 32 days, was reduced by 68% and was not restored by an excess of cytochrome *c* (2, 7).

Comparable Values for the Oxidation of Succinate by Muscle from Adult Males and Females of P. americana, T. molitor, and S. bullata

Table I indicates that the most active system, per milligram of tissue nitrogen, was found in *T. molitor*, that intermediate activity occurred in males of *P. americana* and in both sexes of *S. bullata*, and that activity was lowest in *P. americana* females. The values for activity show a greater difference between species when calculated on a nitrogen content than on a wet weight basis.

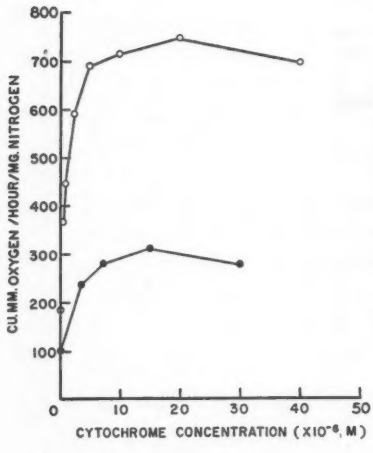


FIG. 3

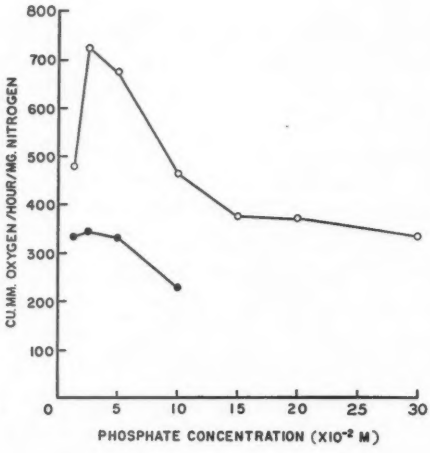


FIG. 4

FIG. 3. The effect of cytochrome *c* concentration on the oxidation of succinate at 30° C. by cockroach muscle. 0.05 *M* phosphate buffer at pH 7.4. Open circles, male, 0.025 *M* sodium succinate. Numbers of determinations, points left to right: 4, 2, 4, 4, 4, 4, 4, 4. Average nitrogen value used. Solid circles, female, 0.05 *M* sodium succinate. Numbers of determinations, points left to right: 2, 2, 5, 5, 4. Nitrogen determined.

FIG. 4. The effect of phosphate concentration on the oxidation of succinate at 30° C. by cockroach muscle. Cytochrome *c* 7.5×10^{-6} *M*, pH 7.4. Open circles, male, 0.025 *M* sodium succinate. Numbers of determinations, points left to right: 7, 7, 8, 8, 4, 2, 2. Average nitrogen value used. Solid circles, female, 0.05 *M* sodium succinate. Numbers of determinations, points left to right: 4, 4, 6, 4. Nitrogen determined.

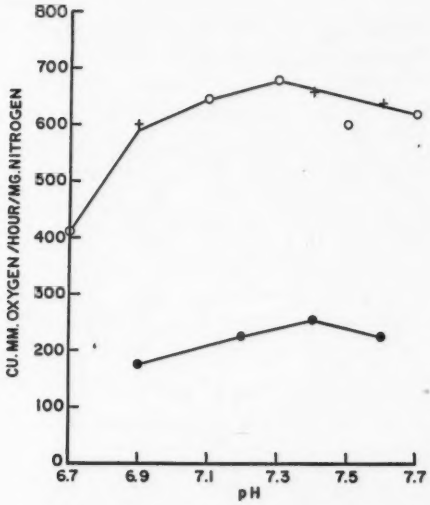


FIG. 5. The effect of hydrogen ion concentration on the oxidation of succinate at 30° C. by cockroach muscle. 0.05 *M* phosphate buffer; 7.5×10^{-6} *M* cytochrome *c*. Open circles, male, 0.025 *M* sodium succinate. Four series of duplicate determinations for each point. Average nitrogen values used. Crosses, the same, except sodium succinate 0.05 *M* and determinations duplicate. Solid circles, female, 0.05 *M* sodium succinate. Numbers of determinations, points from left to right: 6, 3, 7, 6. Nitrogen determined.

TABLE I

DATA ON NITROGEN CONTENT OF MUSCLES AND THE OXIDATION OF SUCCINATE AT 30° C.

(Concentrations of components optimum. Standard deviations shown for all values. Number of cockroaches or groups of beetles and flies averaged, shown in parentheses.)

	Days in adult stage	Mgm. N per 10.0 mgm. of wet muscle	Cu. mm. O_2/hr. per	
			Mgm. N	Mgm. wet wt.
P. americana	10–20			
Male		0.224 ± .037 (14)	648 ± 173 (14)	14.5 ± 4.8 (14)
Female		0.201 ± .036 (12)	167 ± 43 (12)*	3.4 ± 1.2 (12)
P. americana	95–185			
Male		0.258 ± .033 (6)	638 ± 168 (6)	16.2 ± 3.8 (6)
Female		0.227 ± .031 (7)	172 ± 50 (7)*	3.8 ± 0.8 (7)
T. molitor	15–30			
Male		0.151 ± .011 (10)	963 ± 209 (10)	14.6 ± 3.4 (10)
Female		0.141 ± .011 (7)	1115 ± 151 (8)	15.5 ± 2.4 (8)
S. bullata	4–8			
Male		0.193 ± .014 (10)	778 ± 127 (9)	15.0 ± 2.2 (9)
Female		0.177 ± .023 (8)	814 ± 135 (5)	15.6 ± 3.2 (5)

* *Difference between males and females significant at 1% level.*

A three- to four-fold difference in activity between males and females of *P. americana* was highly significant. But significant differences were not found between the two age groups studied.

TABLE II

EFFECTS OF AGE, MUSCLE DEVELOPMENT, AND NITROGEN CONTENT ON OXIDATION OF SUCCINATE BY THE THORACIC MUSCLE OF *Sarcophaga bullata* AT 30° C.

(Buffer 0.05 *M* phosphate at pH 7.4, 0.05 *M* succinate, $7.5 \times 10^{-6} M$ cytochrome *c*. Duplicate determinations. Nitrogen determined for each lot (10 to 14) of flies.)

Age, days	Mgm. wet muscle per fly	Male Mgm. N per 7.5 mgm. wet muscle	$Q_{O_2}(N)$	Mgm. wet muscle per fly	Female Mgm. N per 7.5 mgm. wet muscle	$Q_{O_2}(N)$
Series 1						
4	19.0	0.163	704			
7	22.0	0.163	745	18.0	0.152	748
8	20.0	0.142	924			
9	24.0	0.146	718			
Series 2						
1	6.3	0.137	378	5.0	0.113	375
3	14.3	0.137	279	14.3	0.140	261
5	19.2	0.148	622	21.2	0.119	779
6	20.0	0.132	729*	—	—	—
8	20.0	0.142	924	19.0	0.148	626
9	23.3	0.150	725	16.6	0.103	601

* *Quadruplicate determination.*

In *S. bullata* there was no sex difference but activity increased with adult age. The data obtained from two lots of flies (Table II) show that activity increased from a low level three days after emergence to a peak five days later. The weight of thoracic muscle obtained from each lot of flies was used as a rough measure of muscle development. A three- to four-fold increase in muscle weight occurred in the first five days after emergence, pigmentation of the muscle also increased, but there was not a concomitant change in the nitrogen percentage of the tissue.

Neither sex nor age differences were observed between adults of *T. molitor*.

Discussion

The conditions optimal for the oxidation of succinate by insect muscle homogenates were found to be similar to those recorded for homogenates of mammalian tissue (16) and for oyster muscle preparations (6).

Harvey and Beck (4) used a different general procedure to study the succinoxidase system of male cockroach muscle and found conditions of assay,

at 38° C., similar to those reported in this paper. They found that activity was stimulated by calcium and aluminum ions, at higher concentrations than those required for mammalian systems, especially when phosphate concentration was low. In a few tests completed, increased activity was not observed when calcium and aluminum ions were added, each at 4.0×10^{-4} *M*, to *P. americana* and *T. molitor* muscle homogenates; but the phosphate concentration was optimal. Perhaps homogenates prepared with cockroach saline have different physical properties. The cations were not routinely used, but the comparisons of biological interest observed in this study would probably not be modified by minor differences in technique.

The inhibition by nicotinamide and coenzyme I and partial antagonism of this inhibition by calcium ions found with *P. americana* muscle homogenates, agree with data for mammalian systems (7).

It has been shown (Table I) that differences in the activity of the succinoxidase system that have possible biological significance may not be apparent unless activity is measured in terms of tissue nitrogen. Variations in the activity of the systems from the species studied were not observed when activity was based on the wet weight of muscle.

Alternatively, the sex difference previously reported (1, 4) for *P. americana* is of the same order when calculated on either basis. From this it is evident that the differences in activity are not attributable to water content, fat, or other stored materials, because the muscle of the male cockroach contains only about 10% more nitrogen than that of the female. The results support directly the view of Barron and Tahmisian (1) that muscle of males contains more of the succinoxidase system than females: for although the higher activity of male muscle is associated with greater pigmentation (15) and a higher cytochrome *c* content (1), the sex difference existed when both systems were saturated with cytochrome *c* (Fig. 3). This suggests that the enzyme succinic dehydrogenase or some other component of the system is limiting in female muscle.

Activity differences attributable to age should be considered in terms of tissue nitrogen. In the cockroach, nitrogen content increased about 13% with age (Table I) but activity did not increase. With the fly *S. bullata* (Table II), nitrogen content remained constant for eight days after emergence while the wet weight of muscle and activity increased markedly. This observation suggests that the fraction of protein that is enzymatically active increases in early adult life. More work is needed to clarify the nature of these changes.

This study shows that the succinoxidase system may vary quantitatively among species in the Class Insecta; that differences may occur with adult age, as with *S. bullata*, or there may be no appreciable difference between age groups, as in *P. americana* and *T. molitor*; and that variation may or may not occur between the sexes, as shown for *P. americana* and *T. molitor*, respectively.

References

1. BARRON, E. S. G. and TAHMISIAN, T. N. The metabolism of cockroach muscle (*Periplaneta americana*). J. Cell. and Comp. Physiol. 32 : 57–76. 1948.
2. BODINE, J. H. and LU, K. H. Structure and endogenous oxygen uptake of embryonic cells. Physiol. Zool. 24 : 120–126. 1951.
3. CHADWICK, L. E. and HILL, D. L. Inhibition of cholinesterase by di-isopropyl-fluorophosphate, physostigmine and HETP in the roach. J. Neurophysiol. 10 : 235. 1947.
4. HARVEY, G. T. and BECK, S. D. Muscle succinoxidase in the American cockroach. J. Biol. Chem. 201 : 765–773. 1953.
5. HOPF, H. S. Studies in the mode of action of insecticides. 1. Injection experiments on the role of cholinesterase inhibition. Ann. Applied Biol. 39 : 193–202. 1952.
6. HUMPHREY, G. F. The succinoxidase system in oyster muscle. J. Exptl. Biol. 24 : 352–360. 1948.
7. KEILIN, D. and HARTREE, E. F. Succinic dehydrogenase-cytochrome system of cells. I. Intracellular respiratory system catalysing aerobic oxidation of succinic acid. Proc. Roy. Soc. (London), B, 129 : 277–306. 1940.
8. KEILIN, D. and HARTREE, E. F. Activity of succinic dehydrogenase-cytochrome system in different tissue preparations. Biochem. J. (London) 44 : 205–218. 1949.
9. LORD, K. A. and POTTER, C. Studies of the mechanism of insecticidal action of organophosphorous compounds with particular reference to their anti-esterase activity. Ann. Applied Biol. 38 : 495–507. 1951.
10. MA, T. S. and ZUAZAGA, G. Micro-Kjeldahl determination of nitrogen. Ind. and Eng. Chem., Anal. Ed. 14 : 280–281. 1942.
11. METCALF, R. L. The mode of action of organic insecticides. National Research Council, Washington, D.C. 1948.
12. METCALF, R. L. and MARSH, R. B. Studies of the mode of action of parathion and its derivatives and their toxicity to insects. J. Econ. Entomol. 42 : 721–728. 1949.
13. POTTER, V. R. and ELVEHJEM, C. A. A modified method for the study of tissue oxidations. J. Biol. Chem. 114 : 495–504. 1936.
14. POTTER, V. R. and SCHNEIDER, W. C. Studies on the mechanism of hydrogen transport in animal tissues. V. Dilution effects in the succinoxidase system. J. Biol. Chem. 142 : 543–555. 1942.
15. SACKTOR, B. and BODENSTEIN, D. Cytochrome *c* oxidase activity of various tissues of the American cockroach, *Periplaneta americana* (L.). J. Cell Comp. Physiol. 40 : 157–160. 1952.
16. SCHNEIDER, W. C. and POTTER, V. R. The assay of animal tissues for respiratory enzymes II. Succinic dehydrogenase and cytochrome oxidase. J. Biol. Chem. 149 : 217–227. 1943.
17. SWINGLE, K. F., AXELROD, A. E., and ELVEHJEM, C. A. The mechanism of the effect of calcium salts on the succinoxidase system. J. Biol. Chem. 145 : 581–589. 1942.
18. YEAGER, J. F. Electrical stimulation of isolated heart preparations from *Periplaneta americana*. J. Agr. Research, 59 : 121–137. 1939.
19. ZUKEL, J. W. Some effects of phenothiazine, phenothiazone and thionol on *Periplaneta americana*. J. Econ. Entomol. 37 : 796–808. 1944.

STUDIES ON EFFECTS OF THERMAL CONDITIONING IN TWO SPECIES OF SAWFLY LARVAE[1]

By W. F. Baldwin[2] and H. L. House[2]

Abstract

Conditioning larvae of the sawflies *Neodiprion lecontei* (Fitch) and *Neodiprion sertifer* (Geoff.) at moderately high temperatures increased the thermal tolerance of both species. In both species increased resistance was accompanied by a significant increase in specific gravity of the haemolymph. The osmotic pressure of the haemolymph of *N. sertifer* was increased by about 20%. These effects may be significant in the mechanism whereby increased tolerance to high temperatures is achieved in the living insect. The normal pH of the haemolymph of *N. sertifer* larvae was not altered by heat treatment, indicating that its buffering capacity was not overcome.

Introduction

Animals can acclimate to a wide range of environmental temperatures. The effects of conditioning on the survival of organisms subjected to high temperatures have been studied by many workers. The thermal tolerance of tadpoles reared at high temperatures was increased over that of tadpoles reared at low temperatures (Davenport and Castle (5)). By means of prior thermal treatment the heat–death temperature of black bass was raised 8° C. (Hathaway (8)); the lethal temperature of speckled trout was raised by 2° C. (Brett (4)). More recently, the thermal tolerance for goldfish has been given by Fry, Brett, and Clausen (7).

The heat–death temperatures for insects are subject to modification. Bodenheimer and Klein (3) found that the Palestine ant, *Messor semirufus* E. André, was more resistant to heat in July than in March. It has been shown with chironomids (Walshe (14)) and ephemerids (Whitney (15)) that resistance to extreme temperatures varies with the temperature of the aquatic habitat. Larvae of blow flies reared at high temperatures are more resistant to heat than those reared at low temperatures, and the degree of saturation of phospholipids assimilated in the larvae depends on the temperature (Fraenkel and Hopf (6)). The upper lethal limits and the effects of conditioning over a wide range of temperature have been presented for the chalcid parasite *Dahlbominus fuscipennis* Zett. (Baldwin (1)).

The present data show that resistance of sawfly larvae to high temperatures is increased by thermal conditioning and that this is accompanied by an increase in both the specific gravity and osmotic pressure of the haemolymph.

Methods

The studies were conducted with larvae of two species of sawflies: *Neodiprion lecontei* (Fitch) and *Neodiprion sertifer* (Geoff.). The first experiments were

[1] *Manuscript received October 15, 1953.*
Contribution No. 3125, Entomology Division, Science Service, Department of Agriculture, Ottawa, Canada.
[2] *Agricultural Research Officer, Entomology Laboratory, Belleville, Ontario.*

made with *N. lecontei* in the last feeding instar. These larvae were collected in the field from *Pinus resinosa* Ait. Since the results obtained with these active larvae showed considerable variation, similar experiments were conducted with larvae of *N. sertifer* removed from their cocoons. Larvae of this species were collected from *Pinus sylvestris* L. during the early summer and reared in the laboratory. The development of the larvae within the cocoons was retarded by storage at 1° C. In the present work these larvae are referred to as overwintering larvae, although it is recognized that this species generally passes the winter in the egg stage.

The *N. lecontei* larvae were exposed to moderately high temperatures as follows: immediately after being collected, samples were put in a large flask and submerged in a water bath at 36° C. for 270 min. A stream of air brought as close to saturation as possible was circulated through the flask at 50 cc./min. during treatment. This exposure did not adversely affect the larvae, since control lots of treated individuals continued development and formed cocoons. After removal from the bath, the larvae were held for 24 hr. at 23° C. and a saturation deficiency of 5 gm./cu. meter in a laboratory room.

Tests to determine the resistance of the larvae to high temperatures were conducted as follows: a sample of 200 treated *N. lecontei* larvae was divided equally among 10 glass vials. The vials were connected in series to the air supply and immersed in the water bath at 42° C. The vials were removed at logarithmic intervals over the time range where mortality was known to occur. After 24 hr. at room temperature, the number of dead larvae in each vial was determined. Untreated larvae were used for controls. The experiments were repeated at least six times because of considerable variation within the populations and the results were analyzed by the method of Litchfield and Wilcoxon (10) for relating dose-effect data.

The studies with larvae of *N. sertifer* were conducted in a similar manner except as follows: the larvae, taken from cold storage and removed from their cocoons, were held overnight at 23° C. Conditioning consisted of exposure at 42° C. for 300 min. in the high temperature bath. The tolerance tests were conducted at 45° C. Tests of both control and conditioned samples were completed in duplicate.

As previous work had shown that the specific gravity of the haemolymph of larvae of these sawfly species increased when they were subjected to a temperature of 45° C. (Baldwin and House (2)), the specific gravities of the haemolymph of feeding larvae subjected to the conditioning temperature of 36° C. for various periods were determined to correlate increased resistance with specific gravity. The larvae were exposed to the conditioning temperature in large vials submerged in a water bath operating at 36° C., a stream of saturated air being circulated as before through the vials. The specific gravities of the haemolymph of lots exposed for 30, 60, 90, 120, 150, 180, and 220 min. were determined after removal from the bath. Determinations were made one, two, and three days after heat treatment.

Similar studies were completed with *N. sertifer* larvae. The larvae were removed from the cocoons and held under room conditions for 24 hr. Samples of the larvae were then treated at 42° C. for 30, 60, 90, 150, and 240 min.

The osmotic pressure of haemolymph of *N. sertifer* was determined by the freezing point method. Samples of larvae were removed from the cocoons and held for 24 hr. at room temperature. Treatment consisted of exposure to 42° C. for 150 min. in the constant temperature bath. Droplets of haemolymph were withdrawn from samples consisting of 10 to 15 larvae after a resting period of 24 hr. The haemolymph was pipetted into a very small vacuum flask submerged in a freezing mixture and its freezing point was determined by means of a thermocouple (Robinson (12)). Untreated larvae were used for controls.

The pH of the haemolymph of treated and untreated *N. sertifer* larvae was also determined. The values were obtained by means of a glass electrode and a potentiometer with haemolymph from individual larvae.

Results

The results (Fig. 1) show that the tolerance of active larvae of *N. lecontei* to 42° C. is increased by previous exposure to 36° C. Similarly (Fig. 2), the tolerance of overwintering *N. sertifer* larvae to 45° C. is increased by previous exposure to 42° C.

The curves in Figs. 1 and 2 have been compared by means of median effective dose (ED_{50}) estimates. The value for untreated larvae of *N. lecontei*

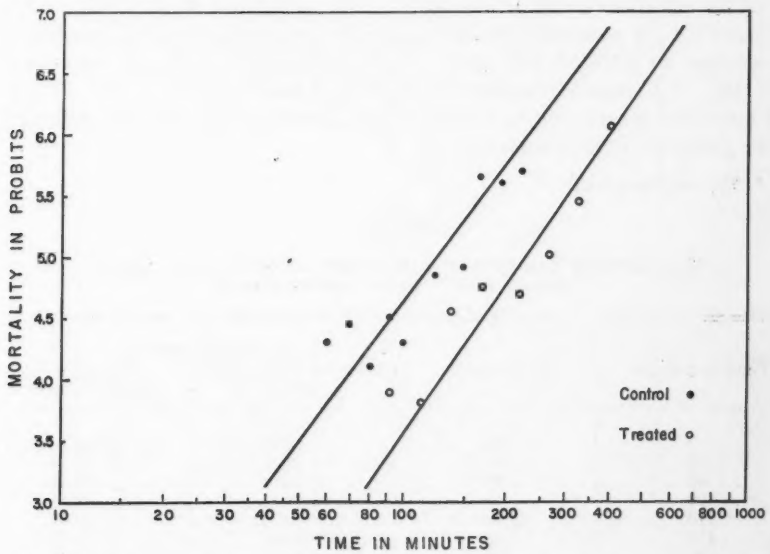


FIG. 1. Resistance of *N. lecontei* larvae in the last feeding stage at 42° C. (●,780 larvae) and after conditioning at 36° C. for 270 min. (○,526 larvae).

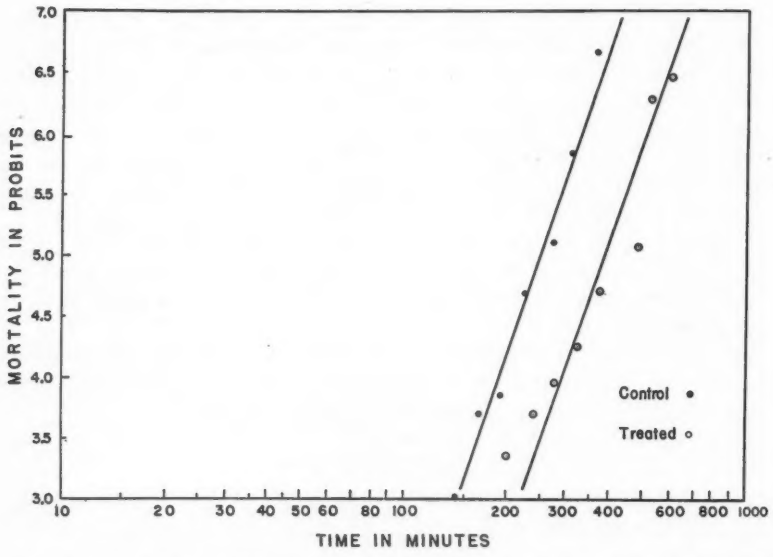


FIG. 2. Resistance of overwintering larvae of *N. sertifer* to 45° C. (●,380 larvae) and after conditioning at 42° C. for 300 min. (○,400 larvae).

held at 42° C. was 125(114–137)* min. in comparison with 220(185–262) min. for larvae previously exposed to 36° C. For untreated larvae of *N. sertifer* held at 45° C. it was 345(231–250) min.; but exposure to 42° C. resulted in an increase to 382(354–412) min. These differences are highly significant ($P < .05$). Chi-square tests showed significant heterogeneity in both treated and untreated samples of *N. lecontei* larvae; heterogeneity was not present in either group of *N. sertifer* larvae.

* *95% confidence limits.*

TABLE I

MEAN SPECIFIC GRAVITIES OF HAEMOLYMPH FROM *N. lecontei* LARVAE EXPOSED TO 36° C. FOR VARIOUS PERIODS

Time in minutes	No. of larvae	Specific gravity	
		Mean	Standard error
0	40	1.0384	± .0070
30	50	1.0447	± .0055
60	49	1.0417	± .0065
90	48	1.0437	± .0089
120	46	1.0431	± .0066
150	46	1.0549	± .0109
180	39	1.0569	± .0086
220	46	1.0553	± .0090

TABLE II

MEAN SPECIFIC GRAVITIES OF HAEMOLYMPH FROM *N. sertifer* LARVAE EXPOSED TO 42° C. FOR VARIOUS PERIODS

Time in minutes	No. of larvae	Specific gravity	
		Mean	Standard error
0	30	1.0541	± .0024
30	20	1.0584	± .0022
60	20	1.0571	± .0017
90	20	1.0602	± .0019
150	20	1.0604	± .0006
240	10	1.0603	± .0028

The specific gravity of the haemolymph was increased in larvae of *N. lecontei* exposed to 36° C. (Table I) and in larvae of *N. sertifer* exposed to 42° C. (Table II) for different periods of time. Similar results were obtained with larvae of *N. sertifer* tested after resting periods of one, two, and three days.

Fig. 3 shows that the increase in specific gravity does not follow a simple curve; a temporary diminution occurs at 60 min. in each species. The increase in specific gravity of haemolymph with exposure to heat is obvious in both species; the change was, however, much greater in feeding larvae of *N. lecontei.*

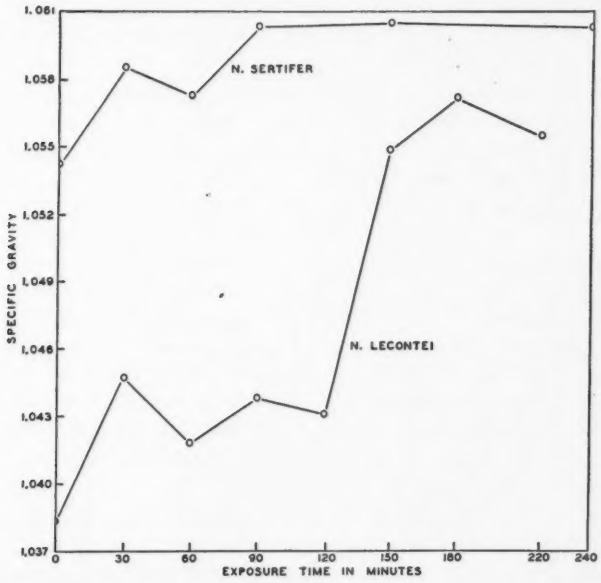


FIG. 3. Specific gravities of haemolymph of overwintering larvae of *N. sertifer* exposed to 42° C. and of larvae of *N. lecontei*, in the last feeding stage, exposed to 36° C. for various periods.

Determinations of the osmotic pressure of haemolymph from untreated and treated larvae of *N. sertifer* showed that the freezing point of the haemolymph was lowered from $-1.16 \pm .03°$ in controls to $-1.39 \pm 0.16°$ C. in heat-treated larvae, a highly significant difference ($P < .01$). This represents an increase in osmotic pressure from $14.0 \pm .01$ to $16.8 \pm .20$ atmospheres, or about 20%.

Tests of the pH of haemolymph from *N. sertifer* larvae exposed to 42° C. revealed that high temperature did not cause any significant change.

Discussion

This work has shown that these sawfly larvae can be acclimated to lethal high temperatures. Associated with this phenomenon was an increase in both the specific gravity and the osmotic pressure of the haemolymph. The increase in haemolymph density in these studies (Fig. 3) was similar to that shown previously (Baldwin and House (2)). The decrease in specific gravity about the 60 min. dosage point was again evident and may have a significant bearing on the response of the regulatory mechanism of the larvae to high temperatures. No change in the pH of haemolymph from treated larvae was noted in this study. This indicates that the buffering capacity was not overcome by the altered properties of the haemolymph. The heterogeneity found in the populations of *N. lecontei* in the tolerance tests may be attributable to the feeding habits of the larvae. In previous work Baldwin and House (2) showed that the degree of desiccation of foliage affects the specific gravity of haemolymph. It is significant that heterogeneity was not found in samples of overwintering larvae of *N. sertifer*.

In insects, two effects of high temperature are increases in specific gravity and in osmotic pressure of the haemolymph as shown in the present study and by Baldwin and House (2). The net change in tonicity of the haemolymph as a result of osmoregulation creates a hypertonic environment for the body cells and may thus increase the resistance of the organism to high temperatures by desiccation of the protoplasm of the body cells. This theory has been advanced by Davenport and Castle (5), who suggested that acclimation to high temperatures may result from a reduction of water in the protoplasm. Vernon (13) has shown that resistance of frog muscle to heat is increased in hypertonic solutions. The effects of increased hypertonicity on the ability of organisms to withstand high temperatures has been reviewed by Heilbrunn (9); he sums up his discussion with the remark that "a decrease in water content tends to retard heat death". Therefore, it appears in our studies with an intact insect that increased tolerance to heat may be directly related to the osmotic homeostasis of the body cells.

In work with mosquitoes, Mellanby (11) has shown that exhaustion of food reserves may be a contributory cause of death at high temperatures. Baldwin (1) found that resistance of unfed females of *Dahlbominus fuscipennis* (Zett.) to lethal high temperatures shows a marked decrease with age. This effect may be attributable to alterations in osmotic balance, since Wigglesworth (16)

has shown that starvation of mosquito larvae causes a decrease in the osmotic pressure of the haemolymph. The decrease in osmotic pressure with starvation indicates that the haemolymph had become hypotonic, and that the body cells may have taken up water, thereby decreasing their resistance to heat.

It is also suggested that changes in the osmotic balance may be concerned in such problems as tolerance to cold. It may be significant that there is a great increase in specific gravity of the haemolymph of these sawflies between the last feeding instar and larvae held as cocoons in cold storage (2). Empirical tests at the Belleville laboratory also have shown that exposure to low temperatures for a short period increased the specific gravity of haemolymph of dipterous larvae. Hence, it appears that acclimation to both heat and cold may be a result of osmoregulation.

References

1. BALDWIN, W. F. Studies on acclimation and lethal temperatures for insects. To be published.
2. BALDWIN, W. F. and HOUSE, H. L. Factors influencing the specific gravity of insect haemolymph. Can. Entomologist, 84 : 131-139. 1952.
3. BODENHEIMER, F. S. and KLEIN, H. Z. Uber die Temperatur Abhangigkeiten von Insekton. II. Die Abhangigkeit der Aktivitat bei der Erteameise *Messor semirufus* E. Andre von Temperatur und anderen Faktoren. Z. wiss. Biol. Abt. C. Z. vergleich. Physiol. 345-385. 1930.
4. BRETT, J. R. Tempering versus acclimation in the planting of speckled trout. Trans. Am. Fisheries Soc. 70 : 397-403. 1940.
5. DAVENPORT, C. B. and CASTLE, W. E. Studies in morphogenesis. III. On the acclimatization of organisms to high temperatures. Arch. Entwicklungsmech. Organ. 2 : 227-249. 1895.
6. FRAENKEL, G. and HOPF, H. S. The physiological action of abnormally high temperatures on poikilothermic animals. I. Temperature adaptation and the degree of saturation of the phosphatides. Biochem. J. 34 : 1085-1092. 1940.
7. FRY, F. E. J., BRETT, J. R., and CLAUSEN, G. H. Lethal limits of temperature for young goldfish. Rev. can. biol. 1 : 50-56. 1942.
8. HATHWAY, E. S. Quantitative study of the changes produced by acclimatization in the tolerance of high temperatures by fishes and amphibians. U.S. Bur. Fisheries Bull. 43 : 169-192. 1928.
9. HEILBRUNN, L. V. An outline of general physiology. 2nd ed. W. B. Saunders Company, Philadelphia and London. 1948.
10. LITCHFIELD, J. T. and WILCOXON, F. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exptl. Therap. 95 : 99-113. 1949.
11. MELLANBY, K. The influence of starvation on the thermal death-point of insects. J. Exptl. Biol. 11 : 48-53. 1934.
12. ROBINSON, W. Determination of the natural undercooling and freezing points in insects. J. Agr. Research, 37 : 749-755. 1928.
13. VERNON, H. M. Heat rigor in cold-blooded animals. J. Physiol. 24 : 239-287. 1899.
14. WALSHE, B. M. The oxygen requirements and thermal resistance of chironomid larvae from flowing and from still waters. J. Exptl. Biol. 25 : 35-43. 1948.
15. WHITNEY, R. J. The thermal resistance of mayfly nymphs from ponds and streams. J. Exptl. Biol. 16 : 374-385. 1939.
16. WIGGLESWORTH, V. B. The regulation of osmotic pressure and chloride concentration in the haemolymph of mosquito larvae. J. Exptl. Biol. 15 : 235-247. 1938.

URETHANE-INDUCED VASODILATATION OF THE FROG TADPOLE[1]

By Donald B. McMillan[2] and Helen I. Battle[3]

Abstract

Ten-millimeter tadpoles of the leopard frog (*Rana pipiens*) were subjected to urethane (ethyl carbamate) in concentrations of 11 mM. (0.10%) and 28 mM. (0.25%) for 18 days. Microscopic examination of sections of these tadpoles revealed a vascular distension of the postcardinal sinuses of the pronephros, the sinusoids of the liver, and the capillaries of the internal gills. A method is described for the quantitative expression of this vasodilatation.

Introduction

During the course of an investigation on the effects of urethane (ethyl carbamate) on the early developmental processes of the leopard frog (*Rana pipiens*), it was observed that continuous subjection of stages from the early blastula to the newly hatched larva resulted in an extensive degree of coelomic oedema and vascular stasis. The latter condition was most pronounced in the vitelline plexus and in the postcardinal sinuses of the pronephric region. These findings corroborated those previously described for urethane-subjected zebra fish eggs (1), but differed in that coelomic oedema could be induced at a much later stage in development than in the zebra fish. In more advanced frog larvae, when the external gills had been superseded by the internal gills, subjection to urethane failed to induce coelomic oedema, but histological sections of such tadpoles revealed vasodilatation in highly vascularized organs, more especially the liver, the pronephros, and the internal gills.

The present investigation has been directed toward a determination of the degree of this vasodilatation in tadpoles subjected to subanesthetic concentrations of urethane for prolonged periods.

Materials and Methods

Ten-millimeter tadpoles of *Rana pipiens*, developed following ovulation induced by the pituitary injection method of Rugh (7), were subjected for 18 days to concentrations of 11 millimoles (0.10%) and 28 mM. (0.25%) urethane. These tadpoles, together with corresponding controls, were maintained at 17° C. in a refrigerating incubator in groups of 30 in 1400 ml. of solution and fed ad libitum green algae and dried egg yolk. Bouin's fluid was employed as a fixative. Serial sections of three samples from each solution were prepared at six microns and stained with Heidenhain's iron haematoxylin (Figs. 2-7).

A quantitative estimation of the degree of vasodilatation was obtained by projecting representative sections of these organs onto graph paper and outlining cellular and vascular areas (e.g. Fig. 1). Sets of five drawings were

[1] *Manuscript received August 9, 1953.*
Contribution from the Department of Zoology, University of Western Ontario, London, Ontario, and carried out under a grant from the National Cancer Institute of Canada.
[2] *Graduate Student, University of Western Ontario.*
[3] *Professor of Zoology, University of Western Ontario.*

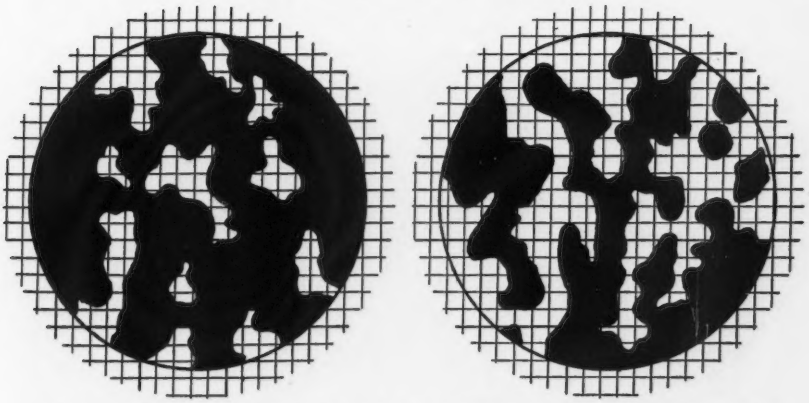


FIG. 1. Silhouette drawings of histological sections of the liver of *Rana pipiens* tadpoles. Hepatic sinusoids are outlined by the trabeculae of hepatic cells which are represented in solid black. The drawing on the right is from a tadpole subjected to 28 mM. urethane for 18 days, that on the left a corresponding control. (× 230).

prepared from different levels of each of the pronephros, the liver, and the internal gills, selected at random from three individuals sectioned from each sample. In these drawings, cellular (tissue) masses were represented in silhouette while vascular areas (blood spaces) were unshaded. The number of squares representing each were totalled and the ratio of vascular area to cellular area was calculated for each drawing. Means of these ratios were determined for each of the three organs for both of the experimental concentrations and for the controls (Table I). The number of squares representing the lumina of the pronephric tubules were also counted to ascertain whether any alteration in their relative size had been brought about by the experimental solutions.

Results

Examination of the experimental results in Table I indicates that distension of the vascular beds comprising the postcardinal sinuses of the pronephros, the sinusoids of the liver, and the capillaries of the internal gills of the frog tadpoles has apparently been induced by subjection to urethane.

Such vasodilatation is illustrated by comparison of photomicrographs of histological sections of these organs from control (Figs. 2, 4, 6) and treated larvae (Figs. 3, 5, 7). In each instance the 95% confidence limits of the ratios of vascular to cellular area for sections of the urethane-treated tadpoles (Table I) are in a range greater than that of the controls. For both the pronephros and the liver, the mean vasodilatation was greater in the 28 mM. than in the 11 mM. solution.

Vascular stasis such as that which occurred extensively in the oedematous newly hatched larvae was not apparent. Lack of such stasis may be attributable to the more complete development of the circulatory system coincident with the advanced degree of organogenesis.

TABLE I

VASCULAR/CELLULAR RATIOS IN ORGANS OF TADPOLES OF *Rana pipiens* AFTER 18 DAYS' SUBJECTION TO URETHANE

(Figures in brackets represent 95% confidence limits)

Concentration of urethane, in mM.	Pronephros	Liver	Internal gills
0 (Control)	0.15 (0.02 – 0.28)	0.22 (0.14 – 0.30)	0.39 (0.22 – 0.56)
11	0.39 (0 – 0.96)	0.29 (0.21 – 0.37)	0.98 (0.79 – 1.17)
28	0.42 (0.40 – 0.44)	0.47 (0.24 – 0.70)	0.80 (0.49 – 1.12)

There was no significant change in the relative size of the lumina of the pronephric tubules consequent upon subjection to urethane. The ratios of areas of tubule lumina to total organ tissue were as follow:

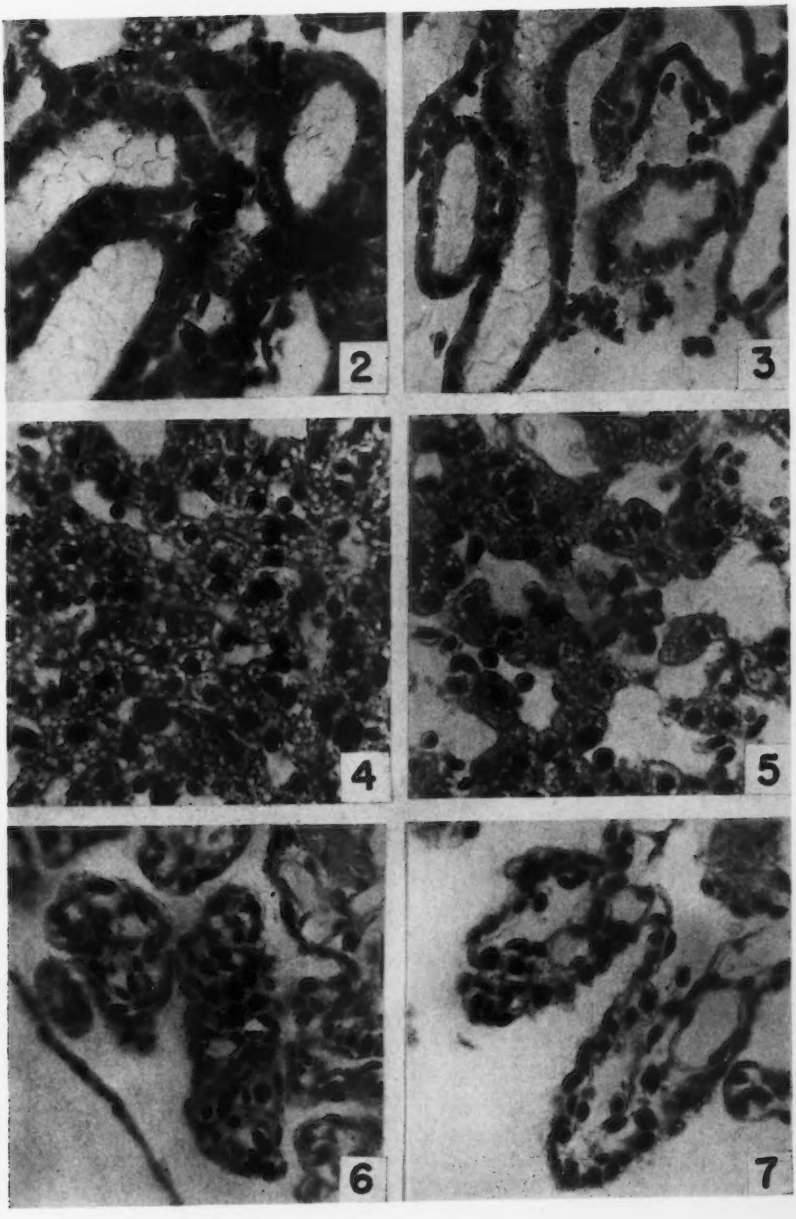
	Mean	95% Confidence limits
Control	0.36	0.16 – 0.56
11 mM.	0.39	0.28 – 0.50
28 mM.	0.35	0.16 – 0.55

No cytological or histological anomalies were evident on detailed microscopic examination of the sections of these tadpoles.

Discussion

Several investigators have demonstrated that urethane exerts an effect on vascular permeability. Krogh and Harrop (3) attributed the dilatation and stasis of the capillaries of the frog tongue after local applications of 5 and 25% urethane to a rapid loss of plasma as a result of changes in the permeability of the capillary walls. Landis (4) suggested that urethane is toxic to the endothelial cells in capillaries of the frog mesentery when injected in stasis-producing concentrations, and that increased permeability produced by the narcotic may be due to injury of the capillary wall. Urethane-induced

FIGS. 2-7. Photomicrographs of histological sections of 26-day-old tadpoles of *Rana pipiens*, six microns in thickness, and stained with Heidenhain's iron haematoxylin. × 300.
FIG. 2. Pronephros of control tadpole. Fig. 3. Pronephros of tadpole reared for 18 days in 28 mM. urethane showing distended postcardinal sinuses. FIG. 4. Liver of control tadpole. FIG. 5. Liver of tadpole reared for 18 days in 28 mM. urethane showing distended hepatic sinusoids. FIG. 6. Internal gill of control tadpole. FIG. 7. Internal gill of tadpole reared for 18 days in 28 mM. urethane showing distended arterial capillaries.


2
3
4
5
6
7

pulmonary oedema in various mammals has been reported by Lushbaugh and Storer (5), Winchester and Higgins (8), and Rosin (6). Winchester and Higgins (8) noted evidences also of capillary damage grossly throughout the body in leukemic mice consequent upon intraperitoneal injections of urethane. Enlarged capillaries filled with erythrocytes, and extravasated plasma surrounding the bloods vessels was recorded by Rosin (6) for the lungs of rats following urethane treatment. Kirschbaum and Bell (2) noted the induction of glomerular lesions in mice by urethane. With the subanesthetic concentrations used in the present experiments, there was no evidence of lesions in the glomi of the pronephros.

In the present paper, an arbitrary vascular to cellular area ratio for histological sections has been used to establish the degree of vasodilatation induced by subanesthetic doses of urethane. Such dilatation has been demonstrated for the arterial capillaries of the internal gills and the venous sinusoids of both the liver and the pronephros of the frog tadpole. The degree of vasodilatation for the latter compact organs was greater in the higher concentrations than in the lower. That such a relationship did not hold for the gills may be attributable to a greater sampling error in this diffuse organ.

The oedema of the pericardial and peritoneal coelom, and the vascular stasis in the vitelline plexus and postcardinal sinuses of the pronephric region induced by urethane in the early frog larva correspond to the condition described by Battle and Hisaoka (1) for the developing zebra fish. In later tadpoles, subjection to urethane results in an accumulation of fluid in the vascular beds rather than in the coelom. This result may be due to the relatively greater vascularization of older tadpoles as compared with that of young larvae. The pronephros, liver, and internal gills appear to be particularly susceptible to such dilatation because of their high degree of vascularization.

Acknowledgment

The authors are indebted to Mr. Charles Jarvis of the University of Western Ontario, Department of Microscopic Anatomy, for the preparation of the photomicrographs.

References

1. BATTLE, H. I. and HISAOKA, K. K. Effects of ethyl carbamate (urethan) on the early development of the teleost *Brachydanio rerio*. Cancer Research, 12 : 334-340. 1952.
2. KIRSCHBAUM, A. and BELL, E. J. Induction of renal glomerular lesions by urethane in inbred mice susceptible to spontaneous glomerulonephritis. Proc. Soc. Exptl. Biol. Med. 64 : 71-72. 1947.
3. KROGH, A. and HARROP, G. A. Some observations on stasis and oedema. J. Physiol. 54 : 125-126. 1921.
4. LANDIS, E. M. Micro-injection studies of capillary permeability. I. Factors in the production of capillary stasis. Am. J. Physiol. 81 : 124-142. 1927.
5. LUSHBAUGH, C. C. and STORER, J. B. Synergistic necrotizing action of urethane and chloroform. Arch. Pathol. 45 : 494-502. 1948.
6. ROSIN, A. Early changes in the lungs of rats treated with urethane (ethyl carbamate). Cancer Research, 9 : 583-588. 1949.
7. RUGH, R. Experimental embryology, a manual of techniques and procedures. New York University Bookstore, New York. 1941.
8. WINCHESTER, W. W. and HIGGINS, G. M. Pulmonary edema in leukemic mice following treatment with urethane. Science, 107 : 568-569. 1948.

STUDIES ON THE ENDOPARASITIC FAUNA OF TRINIDAD MAMMALS

X. PARASITES OF CHIROPTERA[1]

By Robert W. Wolfgang[2]

Abstract

A new genus and species of nematode, *Biacantha desmoda*, subfamily Strongylacanthinae Yorke and Mapleston, 1926, is described from the vampire bat, *Desmodus rufus* Wied, 1826.

Five entire nematodes and three odd fragments from the vampire bat (*Desmodus rufus* Wied, 1826) in Trinidad were found to constitute a species new to science. This material was collected by Prof. T. W. M. Cameron in the British West Indies in 1932. The author is grateful to Professor Cameron for making this material available for study. The parasite check list for Chiroptera (Stiles and Nolan (1)) lists only two parasites of *D. rufus:* a flea (*Trichobius parasiticus*) from Guiana, and a protozoan (*Grahamella brumpti*) from Peru. A review of the literature indicates that no helminths have been described from this host.

Biacantha desmoda gen. et sp. nov.

Specific Diagnosis

Small worms, males, and females are about the same size. The cuticle is marked with longitudinal lines and fine transverse striations. Two complete males were available together with three posterior fragments. The males measure 6.76 to 6.94 mm. in length by 0.23 mm. in maximum width. The three females measure 6.57 to 8.06 mm. in length by 0.20 to 0.23 mm. in width. The esophagus is claviform and measures 0.62 to 0.65 mm. in length, with the nerve ring and the excretory pore at about 0.09 to 0.10 mm. from the anterior end. The terminal mouth is round and measures about 5μ in diameter. There is a cephalic cuticular expansion which measures 0.03 to 0.04 mm. in length by 0.04 mm. in width in the female (Fig. 12) and 0.05 to 0.06 mm. in length by 0.09 mm. in width in the male (Fig. 11). Two posteriorly directed hooks rise from the ventral surface at the posterior limit of the cephalic cuticular expansion and measure 0.03 mm. in the female and 0.02 mm. in the male. A subcuticular swelling which corresponds in position to the hooks can be seen in male specimens.

The male terminates posteriorly in a triangular bursa of the following ray formula (Figs. 5 and 6): ventrals arising from a common trunk; ventroventral not reaching the margin of the bursal membrane and directed cephalad; lateroventral separated from the ventroventral extending parallel and close

[1] *Manuscript received October 14, 1953.*
Contribution from the Institute of Parasitology, McGill University, Macdonald College, P.Q., Canada, with financial assistance from the National Research Council of Canada.
[2] *Research Assistant.*

to the laterals and reaching the margin of the bursal membrane; laterals arising from a common trunk and extending parallel proximally to the bursal margin: the posteriolateral is the largest with the mediolateral being second largest; dorsals arising from a common trunk, the externodorsal projects at an angle to either side of the dorsal and almost reaching the edge of the bursal membrane: the dorsal is divided distally into two lateral branches near its tip with the medial forks bifurcating again at the tips.

The equal spicules are alate, measure 0.16 mm., and are divided distally into two points (Fig. 9). The sclerotization of the spicules is delicate; alae are transparent. The gubernaculum is curved and measures 0.10 mm. in length (Fig. 10). The cloaca is borne on the tip of a genital cone which protrudes into the bursal cavity.

The vulva is situated in the posterior half of the body and is variable in position. It is found at from one third to one fourth the distance from the tail to the head. The female tail (Fig. 8) measures 0.18 to 0.23 mm. and terminates in a spike which is surrounded by three digitiform appendages, one dorsal and two subventral. The appendages measure 0.03 to 0.04 mm. in length. The amphidelphis ovejector (Fig. 7) is composed of two muscular sphincters measuring about 0.07 to 0.08 mm. at either end of a short Y-shaped vagina which leads into a salient vulva orifice. The eggs are few in number (four to six per female) and large, measuring 0.10 to 0.17 mm. in length and 0.08 to 0.14 mm. in width.

Type host: Desmodus rufus Wied, 1826.

Type location: Intestine.

Type locality: Trinidad, B.W.I.

The name *desmoda* is for the host.

Type specimens are to be deposited in the Helminthological Collection of the U.S. National Museum.

The genus *Biacantha* belongs to the subfamily Strongylacanthinae Yorke and Maplestone, 1926, which comprises trichostrongylid genera from bats and edentates. All trichostrongyles so far recorded from chiroptera fall into this subfamily (Travassos (2)). *Biacantha* differs from other genera in the subfamily in possessing cuticular hooks originating in the ventral hypodermis of the head. *Strongylacantha*, which it resembles most closely, differs from *Biacantha* in that it has hooks arising from the mouth (Figs. 1 and 2), a wide subventral mouth, a slightly different bursal ray arrangement (Figs. 3 and 4), and lacks a cephalic cuticular expansion. Table I shows the comparative morphological characters and Figs. 1–4 are a comparison of the contrasting development of cephalic and tail structures in the two genera.

There appears to be a difference in the shape of the cephalic cuticular expansion of male and female worms, but as the description is based on a limited amount of material, a larger series might show this character to be more variable. Figs. 11 and 12 show this difference. The bats are not heavily infected. Professor Cameron's records reveal that only 6 of 31

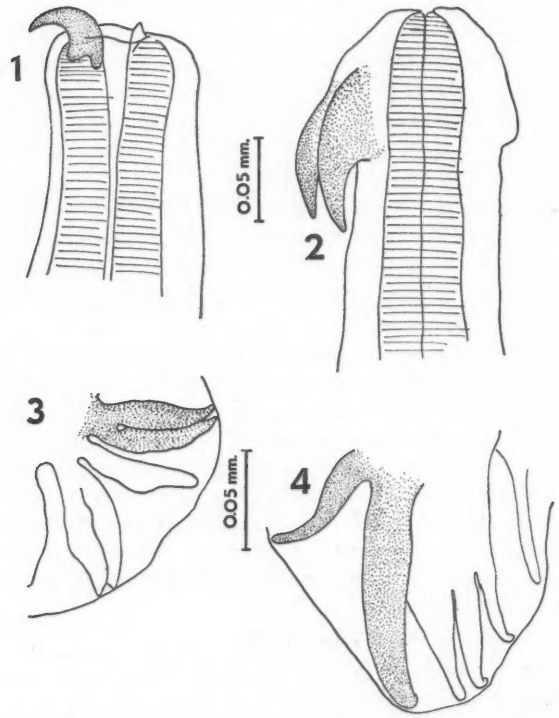


FIG. 1. Head of *S. pretoriensis* Ortlepp, 1932 (original). FIG. 2. Head of *B. desmoda*. FIG. 3. Bursal rays (emphasis on ventrals) *S. pretoriensis* after Travassos (2). FIG. 4. Bursal rays (emphasis on ventrals) *B. desmoda*.

TABLE I

	Cephalic cuticle	Mouth	Hook origin	Ventral rays
Strongylacantha	Noninflated	Large	In the mouth	Parallel, ventroventral directed laterally
Biacantha	Inflated into a cuticular expansion	Small	In hypodermis poststomal	Divergent, ventroventral directed cephalad

vampire bats examined contained worms and only four of these contained *B. desmoda*. Three of the hosts bore single worms and one, a multiple infection.

Strongylacantha occurs in Africa, Eurasia, and Japan in a variety of hosts with a wide distribution. Vampire bats, the host of *Biacantha*, are restricted

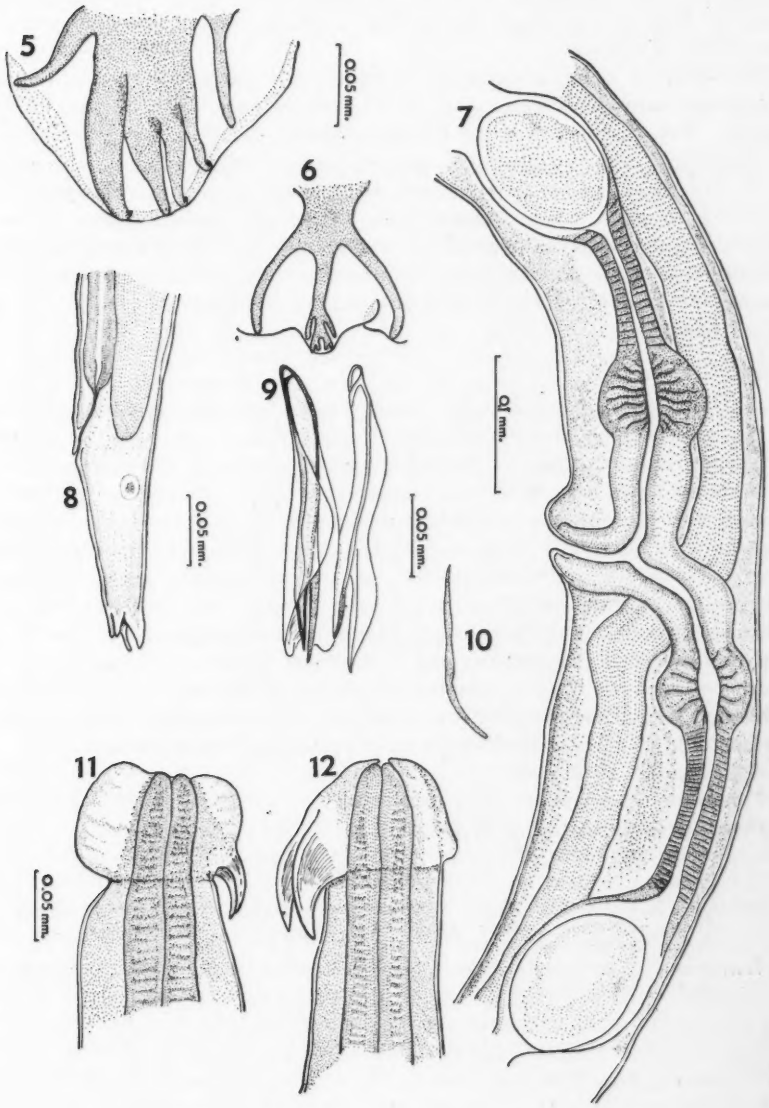


FIG. 5. Bursa (lateral lobe). FIG. 6. Bursa (dorsal lobe). FIG. 7. Lateral view of vulva and ovejector. FIG. 8. Tail of female. FIG. 9. Spicules. FIG. 10. Gubernaculum. FIG. 11. Lateral view of head (male). FIG. 12. Lateral view of head (female).

in their distribution to tropical South and Central America extending north to southern Mexico. It would appear then that the relationship between the two genera is remote.

Females of *B. desmoda* are not present in large numbers according to our record and individuals were never found to have more than six eggs in the uterus. This suggests that the life cycle of this helminth is very efficient. Further, there were two other specimens of this bat which harbored a capillarid worm which has not yet been identified. The presence of a trichostrongyle and a capillarid in a host whose feeding habits are considered strictly confined to lapping blood is puzzling and suggests a need for a more complete investigation of the host's habits. In addition, the lack of information concerning the helminth fauna of this host makes more extensive collection of worms desirable.

Generic Diagnosis

Subfamily Strongylacanthinea. Small worms possessing hooks originating in the ventral hypodermis of the poststomal region of the head. Cephalic cuticular expansion present. Mouth terminal, nerve ring and excretory pore at the same level. Bursa of male triangular with the following ray formula: dorsals with a common stem, dorsal divided distally into two forks, externodorsal similar in length to the dorsal; laterals parallel and proximal arising from a common stem; ventrals divergent and arising from the same stem, lateroventral, parallel and proximal to laterals, ventroventral extends anteriad. Spicules equal, delicately cuticular, and alate. Gubernaculum curved and long. Vulva of female in the posterior half of the body, vulva salient leading into a short Y-shaped ovejector terminated anteriorly and posteriorly by a muscular sphincter. Eggs large and few in number, uteri amphidelphis. Tail of female terminated in a spike with three digitiform processess surrounding it.

Type species: B. desmoda.

Type host: Vampire bat (*Desmodus rufus*).

Distribution: Trinidad, B.W.I.

References

1. STILES, C. W. and NOLAN, M. O. Key catalogue of parasites reported from Chiroptera (bats) with their possible public health importance. Nat. Insts. Health Bull. No. 115. 1931.

2. TRAVASSOS, L. Revisao da familia. Trichostrongylidae Leiper. 1912. Monographias Instituto Oswaldo Cruz No. 1. 1937.

GONOCERCA MACROFORMIS SP. NOV. (DEROGENETINAE: HEMIURIDAE) FROM THE OVARY OF THE COD[1]

By R. W. Wolfgang[2] and B. J. Myers[3]

Abstract

A new species, *Gonocerca macroformis* (Derogenetinae: Hemiuridae) is described from the ovary of the cod taken in Newfoundland waters.

During the summer of 1949 a number of large trematodes were collected from the ovary of the cod, witch, and plaice flounders in Newfoundland waters. These specimens were subsequently sent to the Institute of Parasitology for identification. The authors are indebted to Dr. A. E. Templeman, Director of the Newfoundland Station of the Fisheries Research Board of Canada, for the privilege of examining this material.

The specimens were preserved in formalin and whole mounts were stained with Mayer's paracarmine. Sectioned material for the study of the internal morphology and histology was stained with Delafield's haematoxylin and eosin.

The worms are of a large size and come from an unusual location. A study of 12 specimens indicates that it is a species new to science. The name *macroformis* refers to the large size.

Gonocerca macroformis sp. nov.

Specific Diagnosis

These trematodes are variable in size, attenuated anteriorly and attain their maximum width in the posterior quarter of the body. Length 4.4 to 13.0 mm., width 1.6 to 4.4 mm. The forebody measures 1.6 to 8.0 mm. The oral sucker is subterminal, measuring 0.50 to 0.90 mm. (transverse diameter) and is surmounted by a short preoral lip. The strongly muscular acetabulum measures 0.77 to 1.8 mm. (transverse diameter), and is located slightly posterior to half the distance of the worm in smaller specimens or about half the distance in larger ones. The sucker ratio is 1 : 1.4 to 2.0. The oval muscular pharynx measures 0.1 to 0.25 mm. in diameter and lies immediately posterior to and contiguous with the oral sucker. There is no oesophagus, the caeca branching immediately posterior to the pharynx. Anteriorly, the caeca are thick-walled and composed of compact, columnar epithelium. The cells are much vacuolated and nuclei are of two types: compact in which little nuclear detail could be differentiated, and transparent with a marked centrosome; cytoplasmic processes such as have been described for intestinal epithelium for a number of other trematodes extend into the

[1] *Manuscript received October 14, 1953.*
Contribution from the Institute of Parasitology, McGill University, Macdonald College, P.Q., Canada, with financial assistance from the National Research Council of Canada.
[2] *Research Assistant.*
[3] *Graduate Assistant.*

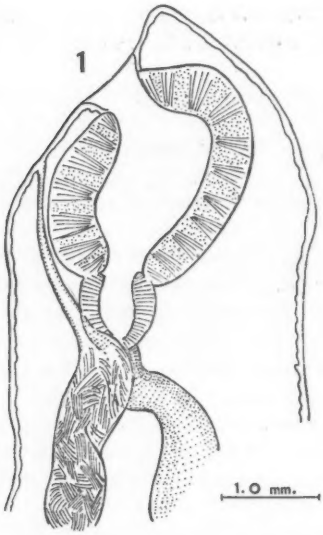


FIG. 1. *Gonocerca macroformis:* anterior end showing subterminal position of oral sucker and location of genital pore.

caecal lumen. Slightly anterior to the acetabulum the caeca become convoluted and broaden to two or three times their anterior width. They extend to the posterior tip of the worm and end lateral to the excretory vesicle but do not unite with it. The lumen of the posterior caeca is broad and the epithelium lining very thin (in most places not more than one cell thick). The histology of this portion of the caeca is similar to that noted for the anterior portion.

The genital pore lies ventral and posterior to the orifice of the oral sucker. There is a short genital sinus followed by the ejaculatory duct and the non-muscular terminal end of the uterus. The seminal vesicle is sinuous, non-muscular, and extends from slightly posterior to the pharynx to a short distance medial to the thick-walled, anterior portion of the caeca. The size and degree of folding of the seminal vesicle varies with the size and contraction of the specimen. The prostatic glands are free in the parenchyma and limited to a short region along the anterior portion of the ejaculatory duct. The uterus is variable in shape and size and extends posteriorly from the genital pore and median to the caeca. Two large vitelline glands composed of compact lobes lie lateral to the ovary. These glands extend over the caeca and are confluent with the lateral margins of the worm. A vitelline duct extends medially from either gland uniting dorsal to the ovary. The small spherical or lobed ovary lies midventrally in the posterior region of the body anterior to the testes and forms the anterior vertex of the gonadal triangle. In contracted specimens it appears to be confluent with the testes

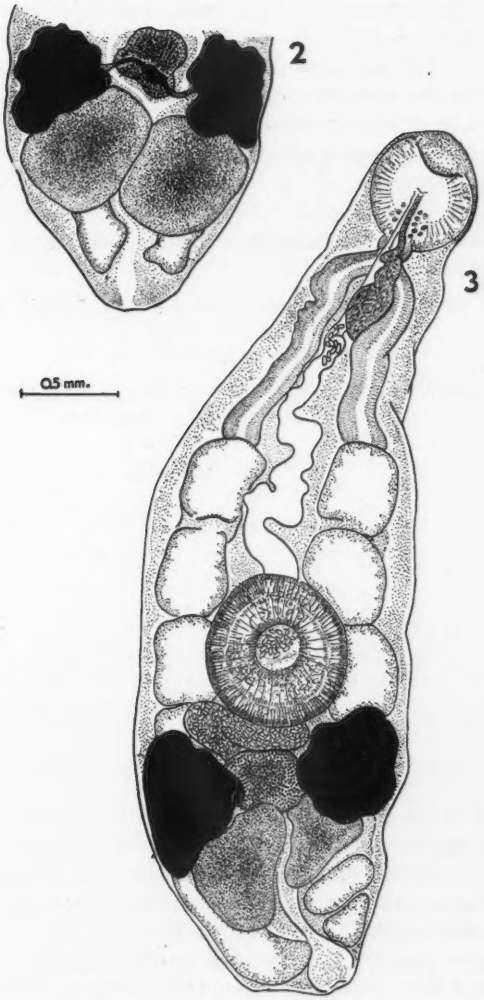


FIG. 2. Posterior end of worm showing testes and ovary with location of the common vitelline duct.

FIG. 3. Entire adult worm. Note variation in position of testes and the difference of breadth of the caeca anteriorly and posteriorly.

but in relaxed specimens it may be seen to be separated from the testes by the lateral branches of the excretory vessels. The testes are about twice the size of the ovary and lie posterior to it and ventral to the caeca. There is a wide terminal excretory vesicle which extends anteriorly to the level of the testes where it branches into two lateral vessels which pass dorsally on either side of the ovary. The eggs are variable in size, 42 to 51 by 21 to 30μ. They are smooth, oval, and nonfilamentous. The cuticle of this worm is smooth although it may be finely wrinkled on the ventral surface.

Type host: Gadus callarius (Cod).

Type location: Ovary.

Type locality: Newfoundland.

Type specimens: to be deposited in the Helminthological Collection, U.S. National Museum.

Table I gives measurements of pertinent organs for eight specimens.

TABLE I

MEASUREMENTS OF PERTINENT ORGANS OF EIGHT SPECIMENS OF *Gonocerca macroformis* SP. NOV.

	Measurements in mm.							
Total length	4.4	7.6	9.0	10.0	10.2	11.0	12.0	13.0
Width	1.6	2.0	3.7	4.0	3.8	4.4	3.8	4.0
Forebody	2.1	4.0	3.8	5.8	6.0	7.0	7.4	8.0
Oral sucker	0.5	0.7	0.7	0.7	0.9	0.8	0.9	0.9
Acetabulum	0.77	*	1.3	1.4	1.0	1.5	1.7	1.8
Egg sizes	44–48 × 21–27μ	42–45 × 27μ	45–51 × 24–27μ	*	42–51 × 27–30μ	*	*	*
Sucker ratio	1 : 1.5	*	1 : 1.9	1 : 2	1 : 1.1	1 : 2	1 : 2	1 : 2

* *Not measured.*

Discussion

Manter (1, 2) erected the genus *Gonocerca* for a species of trematode (*G. phycidis*) occurring on the gills and in the buccal cavity and stomach of the squirrel hake (*Urophycis chuss*) in Maine waters. He discussed the relationship between the genus *Gonocerca* and the genera *Liocerca* and *Hemipera*, two other hiurids with which it was closely related by virtue of a pretesticular ovary. The same author (3) revised his generic diagnosis on the basis of new material found in deep-water fish of the Dry Tortugas, Florida, and added a second species (*G. crassa*) from the local fish of that area.

G. macroformis differs from the previously described species in maximum size, the more attenuated form of the anterior body, and a peculiar construction of the caeca which divides them into two distinct portions—a thick-walled oesophaguslike anterior portion and a broad-lumened thin-walled posterior portion. The uterus is massed mainly ventral to the acetabulum with a single sinuous ascending limb leading to the genital pore.

The location of this worm in the ovary lends a particular significance to its taxonomic position. Structurally, it is similar to those Derogetine hemiurids which inhabit the upper alimentary tract, primarily the stomach of teleost fishes. This species, however, seems to display some of the characteristics of the gorgoderids which undergo a visceral migration in the definitive host. No attempt can be made by the authors to correlate this species with its location in the host, and the fact that it is found commonly in three species of marine fishes which are known to live together in various marine bottoms suggests that it is a true and not an aberrant species. The range of size is remarkable and not unlike similar size ranges demonstrable for tissue stages of some larval trematodes. Whether the gonad has any effect on the growth of the parasite is a matter for conjecture, although it is peculiar that only the ovary seems to have been infected, there being no report or record of specimens from other tissues or the testis.

Gonocerca is an Atlantic genus according to its present array of species. It has not been recorded from European or Pacific waters, although Manter (4) records a closely related genus, *Gonocercella*, from the Galapagos.

The authors have found no previous record of an adult trematode in the gonads of marine fishes. They hesitate to erect a new genus for the present species inasmuch as it fits the characters for the genus *Gonocerca*; some differences are pronounced, however, and include the peculiar construction of the anterior portion of the caeca, the position of the acetabulum with reference to the total length of the worm, the size of the worm, and its location in the host.

References

1. MANTER, H. W. Some marine fish trematodes of Maine. J. Parasitol. 12 : 12–18. 1925.
2. MANTER, H. W. Some North American fish trematodes. Illinois Biol. Monographs, 10 : 1–138. 1926.
3. MANTER, H. W. Some digenetic trematodes from deep-water fish of Tortugas, Florida. Carnegie Inst. Wash. Papers of the Tortugas Laboratory, 28 : 257–345. 1934.
4. MANTER, H. W. Digenetic trematodes of fish from the Galapagos Islands and the neighboring Pacific. Rept. Allan Hancock Pacific Expedition (1932–1938), 2 : 325–497. 1940.

THERMAL TOLERANCE IN *MACROCENTRUS ANCYLIVORUS* ROH. (HYMENOPTERA: BRACONIDAE)[1]

By D. P. Pielou[2] and R. F. Glasser[3]

Abstract

The lethal effects of high temperatures were investigated on *Macrocentrus ancylivorus* Roh., a parasite of the oriental fruit moth. Day-old adults were exposed to uniform high temperatures for fixed periods ranging from 15 min. to 8 hr. in different tests. Temperatures investigated ranged from 30° to 45° C. The insects had been reared under uniform and rigidly specified conditions at constant temperatures. After exposure all the test insects were removed, put in bottles, and provided with 10% sucrose solutions as food. Mortality was recorded 24 hr. later. Sexes were kept separate. Tests at all temperatures and exposure times were carried out both in dry and in moist air. At temperatures up to 34° C. mortality was light even at the 8-hr. exposure. At 44° C. almost 100% mortality was recorded at the 1/2-hr. exposure. Between these limits mortality increased with higher temperature or lengthened exposure time; it was generally greater in dry air than in moist. Females were more resistant than males. Thermograph records from the peach growing areas of the Niagara peninsula showed that only in exceptionally hot summers could conditions cause appreciable death from the direct action of temperature alone.

Methods

In general the method used was to confine insects in watertight vials and pass air of known humidity through these vials at a slow rate while the vials were submerged in a constant temperature bath for a given time.

The water in the insulated bath is heated by a 500 watt flexible tubular heater controlled by a thermostat, accurate to $\pm 0.1°$ C., and a mercury switch relay; two electrical propellor stirrers keep the water in gentle motion and of uniform temperature. A large mass of water, about two cubic feet, aids thermal stability. The experimental vials, 40 in all, are clipped into a carrier framework that can be lifted up and down on guides by a handle. The vials are approximately 16 cm. × 2.5 cm., are closed with rubber stoppers, and are connected in series with glass tubes and rubber connectors. Insects are prevented from passing from one vial to another by small-gauge screens over the air inlet and outlet ports to each vial. The air stream is produced by an electric blower provided with a rate gauge and adjusted to a rate of 100 ml. per min. The air is first filtered through glass wool and then, dependent on whether dry or moist air is required, is passed through either a calcium sulphate gas drying unit (with cobalt chloride indicator) placed outside the

[1] *Manuscript received September 24, 1953.*
Contribution No. 3118, Entomology Division, Science Service, Department of Agriculture Ottawa, Canada.

[2] *Entomology Laboratory, Belleville, Ontario. Present address: Entomology Laboratory, Summerland, B.C.*

[3] *Entomology Laboratory, Belleville, Ontario. Present address: Department of Entomology, Cornell University, Ithaca, N.Y.*

bath or through a Milligan washing bottle filled with distilled water and submerged within the bath. The temperature of the air stream is measured before the air enters the first vial and as it leaves the last one, to check that the temperature is uniform and in equilibrium with the bath temperature. All thermometers used were calibrated against a certified standard thermometer.

The method of lowering or removing the vial rack in one quick elevator motion ensures accuracy and uniformity in achieving the desired period of immersion. A temporary drop in bath temperature had been expected on lowering the cooler carrier and load of vials into the water. To deal with this, a shorting switch had been connected across the relay so that water temperature could be temporarily increased a little, to counteract this expected drop on submersion, without any necessity of altering the thermostat setting. In practice, however, the thermal capacity of the water mass was so overwhelmingly greater than that of the carrier and contents that the fall of temperature was never greater than 0.1° C.

As initial heating of the water mass is slow, a time switch controlling the main power source was set to turn on the heater very early in the morning so that the bath would be at the desired temperature when the experimenter arrived in the morning.

The insects used were all day-old adults reared at a uniform temperature of 26.5° C., by the method of Finney, Flanders, and Smith (3), on the potato tuber moth, *Gnorimoschema operculella* (Zell.), as hosts; they were well fed and separated according to sex. A disk of filter paper was placed at the bottom of each vial. Twenty insects were placed in each of the 40 vials, i.e., 800 insects were used per test. In each test, 20 vials normally contained males, and 20, females.

Insects were handled after exposure to carbon dioxide gas. Carbon dioxide gives almost instantaneous stupefaction and instantaneous recovery on removal from it. After exposures were completed, the insects were placed in standard half pint survival-bottles (7), in which food was supplied as a 10% sucrose solution soaked into cotton dental roll.

Mortality was recorded 24 hr. later; this gave time for insects to recover from any purely knockdown—but nonlethal—effect of high temperature.

In all the definitive experiments recorded here approximately 40,000 insects were used.

The temperatures selected for experimental work were 30.0°, 34.0°, 38.0°, 40.0°, 42.0°, 43.0°, 44.0°, and 45.0° C. The variation of bath temperature and air-stream temperature was within ± 0.1° C. The exposure periods selected were ¼, ½, 1, 2, 4, and 8 hr. Control tests were conducted for each exposure time at 26.5° C., i.e., at the temperature of the constant temperature rooms used in all our experimental and breeding work with *Macrocentrus ancylivorus* adults.

All figures were corrected by Abbot's formula (2).

TABLE I

PERCENTAGE MORTALITIES OF DAY-OLD FEMALE ADULTS OF *M. ancylivorus*

Figures shown are for percentages dead after 24 hr. and are corrected by Abbot's formula. (Upper percentage in each box, mortality in moist air; lower percentage, mortality in dry air)

°C. / Hr.	30°	34°	38°	40°	42°	43°	44°	45°
1/4					6.7 14.4	12.8 5.6	48.4 48.3	62.7 80.9
1/2				5.4 7.8	5.4 34.2	36.9 47.8	96.9 98.0	
1			0.0 0.0	4.5 64.7	81.7 64.2	100.0 99.8		
2	0.0 1.7	4.2 0.0	4.0 0.0	0.0 100.0	100.0 100.0			
4	0.0 1.4	2.0 11.7	2.5 88.6	82.0 100.0				
8	1.4 0.3	10.2 3.4	15.1 100.0	98.6 100.0				

TABLE II

PERCENTAGE MORTALITIES OF DAY-OLD MALE ADULTS OF *M. ancylivorus*

Figures shown are for percentages dead after 24 hr. and are corrected by Abbot's formula (Upper percentage in each box, mortality in moist air; lower percentage, mortality in dry air)

°C. / Hr.	30°	34°	38°	40°	42°	43°	44°	45°
1/4					20.7 0.0	17.0 1.8	63.7 61.6	90.5 84.4
1/2				13.1 0.0	47.5 13.1	72.7 67.6	98.6 100.0	
1			7.8 9.6	28.4 53.2	98.8 86.1	100.0 100.0		
2	2.9 7.9	15.5 2.0	16.7 28.2	23.6 100.0	100.0 100.0			
4	1.5 0.0	12.8 9.3	41.2 100.0	99.8 100.0				
8	25.6 7.7	28.7 25.2	56.6 100.0	100.0 100.0				

Results

The corrected mortality figures, rounded to the nearest 0.1%, are shown in Table I (females) and Table II (males). In the controls there was no increase in mortality with increasing exposure up to 8 hr., and the average control mortalities 24 hr. after completion of tests were: males, moist air, 5.30%; males, dry air, 13.09%; females, moist air, 7.58%; and females, dry air, 10.49%.

These tables show that at temperatures up to 34.0° C. there is no very great mortality even at 8-hr. exposures; males are, however, clearly more susceptible than females. At 38.0° C. high mortality is obtained in dry air to exposures of more than 4 hr. in both sexes; however, in moist air there is low mortality in females, moderate mortality in males. At 40° C. high mortality is obtained in 4-hr. exposures for both sexes in moist air and at shorter exposures in dry air. At these intermediate temperatures in the selected range these relations are apparent: for a given mortality figure there is a rough inverse association between exposure time and temperature; moist air is less lethal than dry air; males are more susceptible than females. These temperatures represent the highest likely to be experienced under Canadian conditions and are therefore the most important ecologically. At still higher temperatures, high mortality is rapidly obtained and these relations, except the inverse relation of exposure time and temperature, tend to be obliterated.

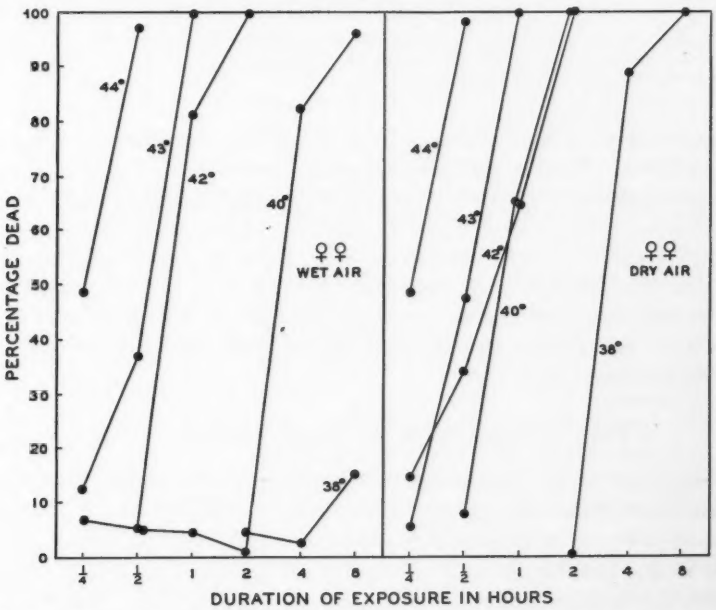


FIG. 1. Effects of increasing exposure time on the survival of populations of females of *M. ancylivorus* at temperatures ranging from 34° to 44° C. Each plotted point is the mean of observations on 400 insects.

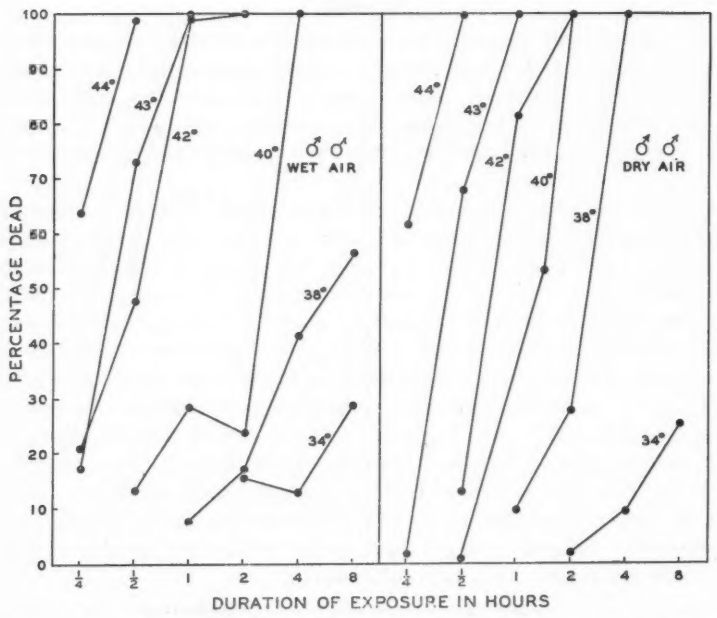


FIG. 2. Effects of increasing exposure time on the survival of populations of males of *M. ancylivorus* at temperatures ranging from 34° to 44° C. Each plotted point is the mean of observations on 400 insects.

Figs. 1 (females) and 2 (males) show graphically the effects of increasing exposure time. Perhaps the best summary of the relations is shown in Fig. 3, which shows smoothed isopleths, drawn like contours on a map, that represent lines of equal mortality—20%, 50%, and 80% (LD_{20}, LD_{50}, and LD_{80})—for the various combinations of temperature, exposure time, and humidity of the air. The modifying effect of moist air, limited to intermediate temperatures only, is apparent, as is also the greater susceptibility and variation in males; particularly the light but very definitely greater mortality of males is noticeable at lower temperatures.

Field Temperatures in Peach Growing Areas

We have examined thermograph records taken under standard meteorological conditions at the Entomology Laboratory, Vineland Station, Ontario.

The records are in degrees Fahrenheit, but a device was constructed by which it was possible to read off rapidly the duration for which a certain temperature in degrees centigrade was exceeded on any day. The durations of high temperatures were listed for the six years 1945–50 and for June, July, and August in each year.

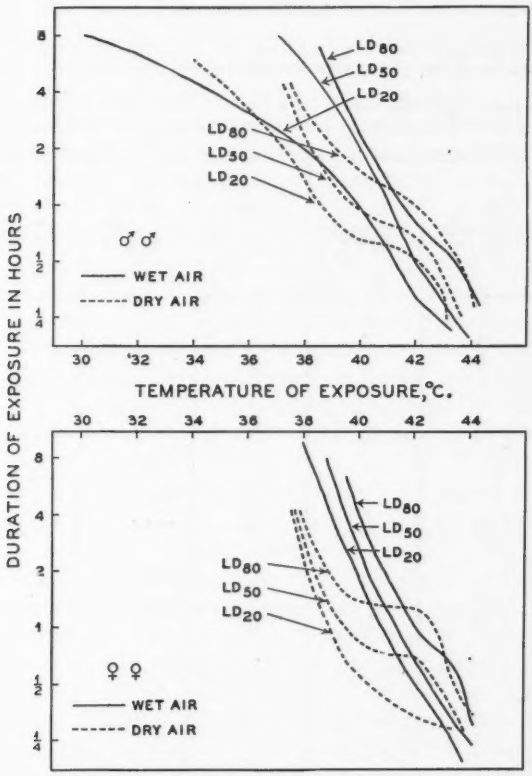


FIG. 3. Smoothed isopleths showing the relation between temperature and exposure time on the survival of *M. ancylivorus*. The lines indicate mortalities of 20%, 50%, and 80% (LD_{20}, LD_{50}, and LD_{80}).

Table III shows the frequencies of periods of such temperatures for the six years. Temperatures are grouped by 2° C. intervals and the durations of the temperatures are grouped geometrically, on a scale of two, in hours.

Unfortunately no figures are available for air humidity. We have noted that dry air is generally more lethal than moist air. If we assume that the air was very dry during these times of high temperature, we get the most pessimistic view of conditions for survival of *M. ancylivorus* populations in this 6-yr. period in the Niagara peninsula of Ontario. Yet a comparison of Table III and Fig. 3 shows that only on very few occasions would natural temperatures, by direct thermal action, cause appreciable mortality. The zone outlined by a heavy line in the lower right of Table III shows those limited periods in the six years when mortality of *M. ancylivorus* females could have exceeded 50% of the female population if conditions did include extreme dryness as well as high temperature. The records in the lower right are almost solely from one very hot year of the six.

TABLE III

FREQUENCIES OF PERIODS OF HIGH TEMPERATURES AT VINELAND STATION, ONTARIO, FOR THE MONTHS OF JUNE, JULY, AND AUGUST IN THE 6-YR. PERIOD 1945–50

The area in the lower right marked off by a heavy line indicates conditions which might cause 50% mortality, or more, in females of *M. ancylivorus*

°C. / Hr.	30.0° and under 32.0°	32.0° and under 34.0°	34.0° and under 36.0°	36.0° and under 38.0°	38.0° and under 40.0°	40.0° and under 42.0°
1/4 and under 1/2	1	4			1	
1/2 and under 1	4	2	1	2		
1 and under 2	12	15	2	1	2	
2 and under 4	38	36	11	2	1	1
4 and under 8	52	23	10	3		
Over 8	24	7	2			

Survival of males under such conditions would be less. However, survival of males is less important as biologically their existence is only necessary during the shorter mating period, whereas the longer oviposition period is important in females; *M. ancylivorus* is, of course, an arrhenotokous parthenogenetic species.

Discussion

In any but very short exposures to high temperatures, dry air is usually conducive to thermal death, because evaporation proceeds rapidly and desiccation is a contributory factor. This is the case in the oriental cockroach, *Blatta orientalis* L., which, in 24-hr. exposures, dies more readily in dry air than in moist (5); and in other but not all insects (6). In our experiments exposures were limited to 8 hr. to reduce the factor of starvation, important in experiments on these fragile parasitic Hymenoptera, as food could not be given conveniently in the apparatus used.

In some, usually larger, insects that have a greater mass of water in their bodies in relation to their surface areas than smaller insects, survival in exposures for very brief periods to high temperatures is higher in dry air than in moist; the insects cool themselves by evaporation for a time so long as water reserves last, and body temperature is then below environment temperature (5, 6). In *M. ancylivorus* there is a bare suggestion of this; and the temperatures concerned are above those encountered under Canadian conditions.

In relating experimental results to field temperatures we can only make comparisons with the temperatures recorded in a screen under standard meteorological conditions. Cooler microclimates may well occur in orchards and aggregation of insects in such microclimates—if they exist—by chance or by behavior response,.would reduce mortality. In other studies, one of us (Pielou) has evidence of a strong behavior response to shallow gradients of temperature in *M. ancylivorus*. It is hoped to continue this work in relation to the data presented in this paper and to observations to be made on thermal conditions in peach orchards.

In fish, thermal death may vary greatly and be related to the previous environmental temperatures (4). Baldwin (1) has investigated the possibility of such acclimation in insects. He has shown that in the hymenopterous parasite *Dahlbominus fuscipennis* Zett. there is evidence of such acclimation but that it is highly complex and influenced by many factors. He concludes also that such acclimation in insects is more apparent than real: that increased or decreased thermal tolerance is influenced by water balance, which in turn is indirectly affected by the conditioning or rearing temperatures. This suggests a wholly different mechanism from the predictable process of thermal tolerance in fish (4). Baldwin's data for day-old parasites acclimated at temperatures ranging from 17° to 29° C. illustrate the complexity of the relation and show that it is difficult to generalize what the effect of previous environmental temperatures will be on thermal death; the same data, however, also show that the quantitative response to acclimation is much less than that shown for fish by Fry, Hart, and Walker (4).

In our own experiments it should be stressed that in making comparisons between field and laboratory data we have been concerned only with the directly lethal effects of high temperature. High summer temperatures may have adverse indirect effects through some change in the environment, e.g., the drying up of water supplies and of sugary plant fluids. Under laboratory conditions, at least, the ready availability of sugar solutions of the right concentration is an important factor in the survival of populations of *M. ancylivorus* (8).

Acknowledgments

We are indebted to Dr. W. F. Baldwin, who has been carrying out a much more extensive study on thermal tolerance in *Dahlbominus fuscipennis* Zett, and other insects, for invaluable advice. The apparatus described in this

paper is a modified form of that used in his studies. We are also indebted to Mr. G. G. Dustan of the Entomology Laboratory, Vineland Station, Ontario, for the loan of thermograph records.

References

1. BALDWIN, W. F. Studies on acclimation and lethal temperatures for insects. Ph.D. Thesis, University of Toronto, 1952.
2. FINNEY, D. J. Probit analysis. Cambridge University Press, London. 1947.
3. FINNEY, G. L., FLANDERS, S. E., and SMITH, H. S. Mass culture of *Macrocentrus ancylivorus* and its host, the potato tuber moth. Hilgardia, 17 : 437–483. 1947.
4. FRY, F. E. J., HART, J. S , and WALKER, K. F. Lethal temperature relations for a sample of young speckled trout *Salvelinus fontinalis*. Univ. Toronto Studies Biol. Ser. 54 : 9–35. 1946. (Publ. Ontario Fisheries Research Lab. 66).
5. GUNN, D. L. and NOTLEY, F. B. The temperature and humidity relations of the cockroach. IV. Thermal deathpoint. J. Exptl. Biol. 13 : 28–34. 1936.
6. MELLANBY, K. The influence of atmospheric humidity on the thermal death point of a number of insects. J. Exptl. Biol. 9 : 221–231. 1932.
7. PIELOU, D. P. and GLASSER, R. F. Selection for DDT tolerance in a beneficial parasite, *Macrocentrus ancylivorus* Roh. I. Some survival characteristics and the DDT resistance of the original laboratory stock. Can. J. Zool. 29 : 90–101. 1951.
8. PIELOU, D. P. and GLASSER, R. F. The survival of *Macrocentrus ancylivorus* Roh., a parasite of the oriental fruit moth, on different concentrations of various sugar solutions. Can. J. Zool. 31 : 121–124. 1953.

Notes to Contributors

Manuscripts

(i) ***General***

Manuscripts should be typewritten, double spaced, on paper 8½ × 11 in. **The original and one copy are to be submitted.** Tables (each typed on a separate sheet) and captions for the figures should be placed at the end of the manuscript. Every sheet of the manuscript should be numbered.

Style, arrangement, spelling, and abbreviations should conform to the usage of this journal. Names of all simple compounds, rather than their formulas, should be used in the text. Greek letters or unusual signs should be written plainly or explained by marginal notes. Superscripts and subscripts must be legible and carefully placed.

Manuscripts should be carefully checked before they are submitted; authors will be charged for changes made in the proof that are considered excessive.

(ii) ***Abstract***

An abstract of not more than about 200 words, indicating the scope of the work and the principal findings, is required, except in Notes.

(iii) ***References***

References should be listed **alphabetically by authors' names,** numbered, and typed after the text. The form of the citations should be that used in this journal; in references to papers in periodicals, titles should be given and inclusive page numbers are required. All citations should be checked with the original articles, and each one referred to in the text by the key number.

(iv) ***Tables***

Tables should be numbered in roman numerals and each table referred to in the text. Titles should always be given but should be brief; column headings should be brief and descriptive matter in the tables confined to a minimum. Numerous small tables should be avoided.

Illustrations

(i) ***General***

All figures (including each figure of the plates) should be numbered consecutively from 1 up, in arabic numerals, and each figure should be referred to in the text. The author's name, title of the paper, and figure number should be written in the lower left-hand corner of the sheets on which the illustrations appear. Captions should not be written on the illustrations (see Manuscripts (i)).

(ii) ***Line drawings***

Drawings should be carefully made with India ink on white drawing paper, blue tracing linen, or co-ordinate paper ruled in blue only; any co-ordinate lines that are to appear in the reproduction should be ruled in black ink. Paper ruled in green, yellow, or red should not be used unless it is desired to have all the co-ordinate lines show. All lines should be of sufficient thickness to reproduce well. Decimal points, periods, and stippled dots should be solid black circles large enough to be reduced if necessary. Letters and numerals should be neatly made, preferably with a stencil (**do NOT use typewriting**), and be of such size that the smallest lettering will be not less than 1 mm. high when reproduced in a cut 3 in. wide.

Many drawings are made too large; originals should not be more than 2 or 3 times the size of the desired reproduction. In large drawings or groups of drawings the ratio of height to width should conform to that of a journal page but the height should be adjusted to make allowance for the caption.

The original drawings and one set of clear copies (e.g. small photographs) are to be submitted.

(iii) ***Photographs***

Prints should be made on glossy paper, with strong contrasts. They should be trimmed so that essential features only are shown and mounted carefully, with rubber cement, on white cardboard.

As many photographs as possible should be mounted together (with a **very** small space between each photo) to reduce the number of cuts required. Full use of the space available should be made and the ratio of height to width should correspond to that of a journal page; however, allowance must be made for the captions. Photographs or groups of photographs should not be more than 2 or 3 times the size of the desired reproduction.

Photographs are to be submitted in duplicate; if they are to be reproduced in groups one set should be mounted, the duplicate set unmounted.

Reprints

A total of 50 reprints of each paper, without covers, are supplied free. Additional reprints, with or without covers, may be purchased.

Charges for reprints are based on the number of printed pages, which may be calculated approximately by multiplying by 0.6 the number of manuscript pages (double-spaced typewritten sheets, 8½ in. × 11 in.) and making allowance for illustrations (not inserts). The cost per page is given on the reprint requisition which accompanies the galley.

Any reprints required in addition to those requested on the author's reprint requisition form must be ordered officially as soon as the paper has been accepted for publication.

Contents

Page

Oxygen Uptake of Muscle Homogenates from Three Species of Insects, in the Presence of Added Succinate, Cytochrome *c*, and Phosphate—*Willard R. Allen and A. Glenn Richards* - - - - - - - - - 1

Studies on Effects of Thermal Conditioning in Two Species of Sawfly Larvae—*W. F. Baldwin and H. L. House* - - - - - - - - 9

Urethane-induced Vasodilatation of the Frog Tadpole—*Donald B. McMillan and Helen I. Battle* - - - - - - - - - - - 16

Studies on the Endoparasitic Fauna of Trinidad Mammals. X. Parasites of Chiroptera—*Robert W. Wolfgang* - - - - - - - 20

***Gonocerca macroformis* sp. nov. (Derogenetinae: Hemiuridae) from the Ovary of the Cod**—*R. W. Wolfgang and B. J. Myers* - - - - - 25

Thermal Tolerance in *Macrocentrus ancylivorus* Roh. (Hymenoptera: Braconidae)—*D. P. Pielou and R. F. Glasser* - - - - - - - 30